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James B. Phillips · David Hercher
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Peripheral Nerve Tissue Engineering and Regeneration



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Reference Series in Biomedical Engineering

Tissue Engineering and Regeneration

Series Editor

Heinz Redl, Ludwig Boltzmann Institute for Traumatology in coop. with AUVA,
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James B. Phillips • David Hercher
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Editors

Peripheral Nerve Tissue Engineering and Regeneration

With 73 Figures and 18 Tables

 Springer

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Preface

Regeneration of peripheral nerves after injury remains a challenge to both patients and clinicians. Even after more than 70 years of microsurgery in this field, the outcome is often poor, and patients may suffer from dysfunction and disability, and also from neuropathic pain. Advances in tissue engineering and regenerative medicine and improved understanding of nerve biology have resulted in new technologies for the treatment of nerve injuries with the potential to improve outcomes for patients.

This book aims to provide an overview of current knowledge in nerve tissue engineering and regeneration, with chapters drawing on expertise in neurobiology, biomaterials, tissue engineering, therapeutics, model systems, surgery, and rehabilitation from an international range of experts. Successful nerve tissue engineering and regeneration requires a multidisciplinary approach, benefitting from research and treatment communities sharing knowledge, ideas, and experiences. With this in mind, the chapters have been prepared to introduce specific areas in an accessible manner, providing a starting point for understanding some of the key challenges and opportunities in this field.

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James B. Phillips
David Hercher
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About the Editors



James B. Phillips is Professor of Regenerative Medicine and Vice Dean (Innovation and Enterprise) in the Faculty of Life Sciences at University College London. He is also co-Director of the UCL Centre for Nerve Engineering and Chief Scientific Officer of the UCL spinout company Galign Ltd. His research focus on translational neuroscience includes construction of living artificial tissues for regenerative medicine; developing novel cell, drug, gene, and biomaterial therapies for neural repair and protection; and advanced 3D co-culture models. Applications include treating and modeling neurodegenerative diseases and traumatic injury to peripheral nerves, the spinal cord, and the brain. His multi-disciplinary research group is based in the Department of Pharmacology at the UCL School of Pharmacy, and uses *in silico*, *in vitro*, *in vivo*, and clinical approaches. Professor Phillips has been involved in nerve tissue engineering and regeneration research for more than 20 years. His lab pioneered Engineered Neural Tissue (EngNT) and is developing other biomaterial and small molecule therapeutics to treat nerve injury, as well as investigating the cellular, molecular, and mechanical properties of nerves.



David Hercher obtained his MSc in molecular biology from the University of Vienna and his Dr. scient. med. from the Medical University of Vienna. Since 2017, he is the head of the Neuroregeneration Group at the Ludwig Boltzmann Institute for Traumatology, where he has worked for 12 years in the field of regenerative medicine. His group focuses its research on the investigation of regenerative processes after injuries to the nervous system and the subsequent development of

novel treatment options for peripheral and central nervous injuries. Furthermore, the group's interests lie in the elucidation of modes of action of mechanical stimuli on neuronal/glial cells and tissues as well as non-viral gene therapy and novel imaging modalities and their application in the field of regenerative medicine.



Thomas Hausner was born in Salzburg, Austria, in 1965. He is Doctor of Medicine since 1993. His specializations in Medicine are General and Visceral Surgery, Trauma Surgery, and Hand- and Microsurgery. Currently he is Head of AUVA Trauma Center Vienna, Lorenz Böhler, Austria, and Co-Director of Ludwig Boltzmann Institute for Traumatology, located at Trauma Center Lorenz Böhler. Postdoctoral Lecture Qualification for Trauma Surgery at Paracelsus Medical University Salzburg in 2015. His main field of research is regeneration of peripheral nerves after trauma. This includes effects of extracorporeal shock wave therapy on nerve regeneration, bridging nerve defects and neural plasticity after injury to the peripheral neural system. Especially bridging nerve defects is a tissue engineering topic, e.g., the development of nerve scaffolds and tubes from silk or different other materials. Furthermore, together with his fellows he is working on various aspects of hand trauma like scaphoid fractures and their delayed healing as well as distal radius fractures. A new field for the research group is the use artificial intelligence through deep learning systems in radio-diagnostic of fractures. Dr. Hausner is also member of the German Nerve Club, an interdisciplinary nerve study group.

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The History of Nerve Repair

Susan Standing

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Abstract

This brief review traces the history of peripheral nerve repair, from the nihilistic attitude of early Greek physicians, via the occasional Renaissance proponent of tension-free anastomosis of nerve stumps, and the centuries-long reluctance of most surgeons to intervene for fear of causing severe postoperative pain, to current clinical practice involving microsurgery. Although the need to treat nerve injuries sustained in battle has long been a major driver in the quest for effective treatment, the transition from empiricism to evidence-based practice has occurred relatively recently along this time line. Modern concepts of the structure of peripheral nerves and their cellular responses to traumatic injury evolved in the nineteenth century *pari passu* with the emergence of increasingly sophisticated microscopical and neurophysiological techniques. Defining the cellular and molecular events that occur after a nerve has been injured, whether by ischemia, crush, or transection, informs the current management of such injuries. Despite many decades of research, it is a sobering thought that functional outcomes after repair frequently remain unsatisfactory.

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1 Introduction

This review begins with a short section that introduces the reader to the organization of the nervous system and the way in which the peripheral nervous system responds to traumatic injury: it is intended to contextualize what follows.

1.1 The Organization of the Nervous System

The two major divisions of the nervous system are the *central nervous system* (CNS) and the *peripheral nervous system* (PNS). The CNS consists of the brain and spinal cord. For the purposes of description, the PNS is conventionally divided into four systems: the cranial and spinal nerves, their sensory ganglia, and their peripheral ramifications, principally within striated muscle, skin, periosteum, bone, tendon, and joint capsules; the peripheral *autonomic nervous system* (ANS) subdivided into the sympathetic and parasympathetic systems that collectively innervate exocrine secretory glands, cardiac muscle, and smooth muscle; the *enteric nervous system* (ENS) composed of ganglionated neuronal plexuses (myenteric and submucosal) in the walls of the gastrointestinal tract that are capable of sustaining local reflex activity independent of the CNS; and the *visceral afferent system* which has vagal and spinal components that convey sensory information to the CNS from the viscera, glands and blood vessels (Gebhart and Bielefeldt 2016).

Depending upon their destination and function(s), peripheral nerves may contain somatic motor and/or sensory axons, and/or populations of autonomic or visceral afferent axons. Motor nerves contain the axons of neurons situated in either discrete nuclei in the brainstem or upper cervical spinal cord (cranial nerves III, IV, V, VII, X, XI and XII) or in the ventral horn of the spinal cord: condensations of motor neurons in the cervical and lumbar ventral horns innervate the muscles of the upper and lower limbs, respectively. Sensory nerves contain the peripheral processes (axons) of sensory neurons with cell bodies in either the sensory ganglia of certain cranial nerves (V, VIII and X) or in segmental dorsal root ganglia. The central processes of these neurons terminate in nuclei in the brainstem (sensory fibers from the face and scalp terminate in the trigeminal nuclei and sensory fibers from the body terminate in the gracile or cuneate nuclei).

1.1.1 The Structure of a Peripheral Nerve

All neurons, irrespective of their size and shape, consist of a cell body (perikaryon, soma) and processes (axons and dendrites): amacrine cells in the retina are unusual in that they lack axons. In the PNS, axons are the long peripheral processes of neurons with cell bodies in either sensory or autonomic ganglia or in the ventral horn of the spinal cord. The glial cell type that populates much of the PNS is the neural crest-derived Schwann cell. Glia in the intrinsic enteric plexuses are transcriptionally distinct from Schwann cells, oligodendrocytes, and astrocytes (Rao et al. 2015). Olfactory ensheathing cells surround bundles of very small axons in the olfactory nerve: these glial cells are heterogenic with regard to their morphology and antigen

expression, sharing phenotypic properties with both Schwann cells and astrocytes (Yang et al. 2015; Pellitteri et al. 2016).

Peripheral axons other than in the ENS and olfactory nerve are associated along their length with Schwann cells, either until they reach their termination or a little before this point when they become naked nerve endings (e.g., in the skin and fascia). In general, axons $>2\text{ }\mu\text{m}$ diameter are myelinated: at any point along their length they have a 1:1 relationship with their ensheathing Schwann cells. The cytoplasmic processes of neighboring Schwann cells interdigitate at nodes of Ranvier along each myelinated axon; internodal length increases with axonal calibre and sheath thickness. Smaller axons, $<2\text{ }\mu\text{m}$ diameter, are ensheathed in groups within longitudinal chains of interdigitating Schwann cells: they may pass between these cellular chains as they pass along a fascicle. All axon – Schwann cell units, whether myelinated or unmyelinated – are surrounded by continuous sleeves of basal lamina secreted by the Schwann cells. At neuromuscular junctions, basal lamina extends between nerve and muscle, projecting into invaginations of the postsynaptic membrane. The functional relationship between axons and Schwann cells is normally tightly regulated by reciprocal signaling: re-establishing that relationship is an essential but not sufficient determinant of successful nerve regeneration. Groups of axons and associated cellular and acellular elements form a fascicle (also called a funiculus). The size, number and arrangement of fascicles vary in different nerves and at different levels along their paths from limb root to digital tips, particularly close to a point of branching (Sunderland 1945a; Williams and Jabaley 1986). At places where nerves are typically subject to pressure during routine movements, e.g., deep to a retaining band or retinaculum at the wrist or ankle, the number of fasciculi increases but their individual size decreases. Each fascicle contains axons and populations of non-neural cells, lying within an endoneurium and enclosed by a multi-layered, collagen-rich cellular sheath, the perineurium, itself surrounded by an epineurium. From a clinical perspective, knowledge of intraneural topography is important when suturing the stumps of transected nerves, placing nerve grafts and planning nerve transfer operations.

Epineurium consists of an outer paraneural layer of loose connective tissue enriched with adipocytes, and an inner interfascicular layer loosely attached to the perineurium. The paraneural layer may function as a shock absorber, protecting the nerve from recurrent compression and allowing it to glide within its tissue bed; the interfascicular layer probably allows individual fascicles to slide independently within the main bundle. In general, the more fasciculi that form a peripheral nerve, the thicker will be the epineurial layers surrounding the entire nerve and lying between individual fascicles (Sunderland 1945b). The epineurium is frequently the layer that is sutured in clinical nerve repair: gross fascicular matching when coapting proximal and distal nerve stumps may be facilitated by matching epineurial blood vessel patterns.

Perineurium extends around each fascicle from the CNS–PNS transition zone to the periphery (Ushiki and Ide 1990), where it either becomes continuous with the capsules of muscle spindles and encapsulated sensory endings or ends openly at unencapsulated endings and neuromuscular junctions. In contrast to the loose

organization of the epineurium, the perineurium is highly structured. It consists of layers of flattened fibroblast-like cells that interdigitate along extensive tight junctions, each layer enclosed by a continuous basal lamina and separated from its neighbor by collagen. Perineurial cells exhibit cytological features consistent with a role as a metabolically active diffusion barrier between epineurium and endoneurium. The perineurium is thought to play key roles in determining the elasticity and tensile strength of an intact nerve and in maintaining a positive intraneural pressure: sudden loss of this pressure, as happens when a nerve is transected, causes the endoneurial contents to protrude like mushrooms beyond the edges of the proximal and distal nerve stumps. When regrowing axons and their associated Schwann cells are unable to access the microenvironment of a denervated distal stump or a nerve graft, perineurial cells grow out from the proximal nerve stump and ensheath the axon-Schwann cell units within vascularized “minifascicles.”

Endoneurium has cellular and acellular components. The former include axons and their associated Schwann cells, endogenous macrophages, fibroblasts, endothelial cells, pericytes, and mast cells. These all lie in a fibrous matrix of fine bundles of type III collagen (reticulin) fibers arranged either parallel to the long axis of the fascicle nerve or condensed around individual Schwann cell–axon units and endoneurial vessels. Blood vessels run obliquely across the perineurium between the epineurium and the endoneurium, and so blood flow may be compromised when nerves are stretched or compressed: even moderate elevations in endoneurial fluid pressure can deform perineurial vessels from cylinders to ellipses, reducing the cross-sectional area of their lumens (Myers et al. 1986; Lundborg 1988; Höke et al. 2001).

Epineurial and perineurial vessels have a dense perivascular plexus of peptidergic, serotonergic, and adrenergic nerves, whereas the smooth muscle layer in endoneurial arterioles is poorly developed and so autoregulation is poor. Free nerve endings in the epineurium, perineurium, and/or endoneurium may contribute to the acute sensitivity of a nerve that has been trapped by a fibrotic scar after injury or surgery.

1.2 Wallerian Degeneration: An Overview

An axon that has been separated from its cell body cannot survive and auto-destructs distal to the injury site (Fig. 1). All affected axons in the extreme tip of the proximal stump and throughout the distal stump of a crushed or transected peripheral nerve degenerate, irrespective of their caliber or functional modality. During the first weeks after injury, the constitutive tissue response involves a cascade of events, some sequential, others consecutive, some occurring locally, and others at a distance from the site of injury. Dissecting the signaling pathways that regulate these events may reveal target molecules for therapeutic intervention.

An axotomized neuronal cell body prepares for axonal regrowth, as evidenced by upregulation of immediate early genes (e.g., c-Jun) and regeneration-associated genes (RAGs) responsible for the expression of cytoskeletal proteins such as actin

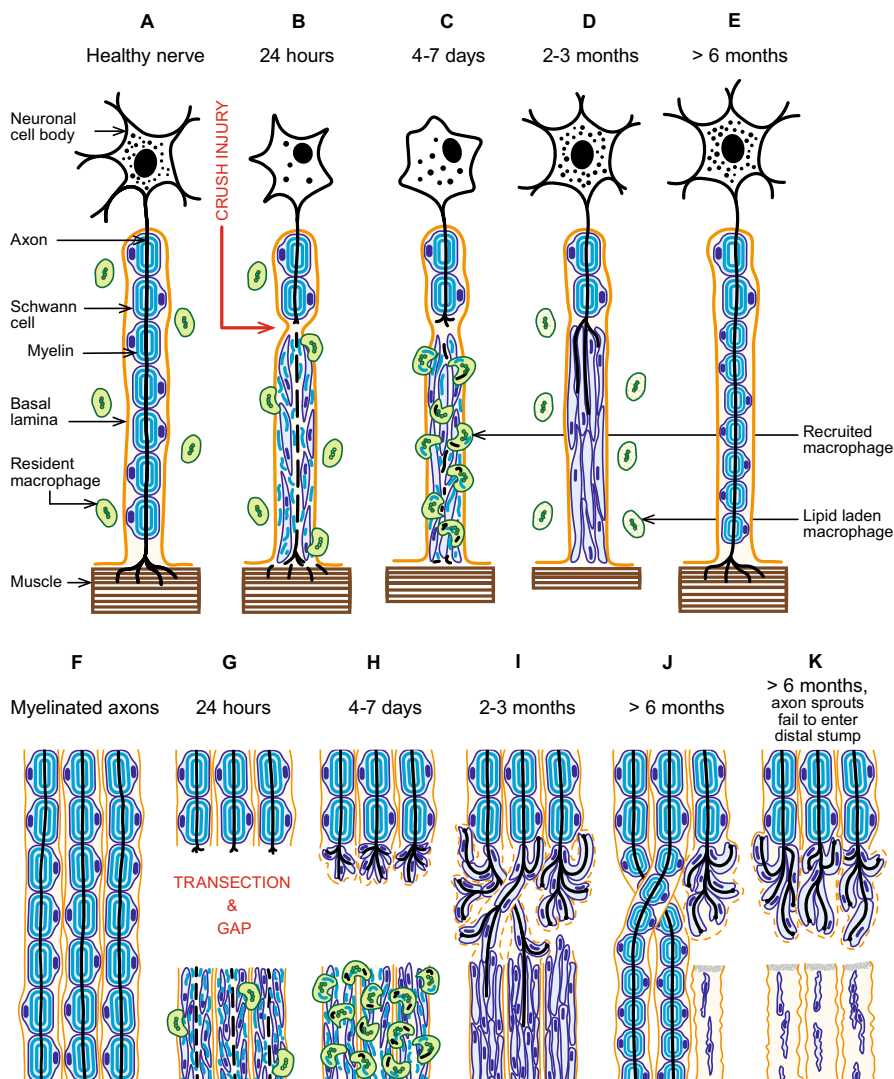


Fig. 1 Overview of Wallerian degeneration. (a–e) events occurring within a myelinated peripheral nerve fiber after crush. (a) normal myelinated axon associated with Schwann cells and enclosed within a continuous basal lamina; (b, c) within 7 days of injury, the axon sprouts from its proximal stump, Schwann cells distal to the injury proliferate, and the axon and all of its associated myelin sheaths distal to the crush site are degraded and phagocytosed by macrophages that have penetrated the basal lamina. The neuronal cell body undergoes significant changes, including up-regulation of immediate early genes (e.g., c-Jun) and regeneration-associated genes (RAGs) that are sometimes recognizable microscopically as chromatolysis. The daughter Schwann cells in the distal segment line up within the basal lamina forming a Schwann tube (band of Büngner); (d, e) ingrowing axonal sprouts establish a relationship with the acutely denervated Schwann cells, are myelinated and may connect with the original end organ, depending on the distance from injury site to target organ. Myelin sheaths are thinner and internodal lengths are shorter than those along the proximal,

and tubulin that are anterogradely transported along the regrowing axons. Genes that transcribe proteins involved in specialized neuronal activities, e.g., choline acetyl transferase, are downregulated. In some, but by no means all neurons, the somata undergo a characteristic series of morphological changes collectively called chromatolysis.

Axonal degeneration, as seen in rodent transected nerves, occurs in distinct morphological stages: timings of each stage may vary in larger animals, but there is no reason to believe that the Ca^{2+} -dependent program that is initiated by injury is essentially different between mice and men. Events occurring during the first 12 h after axotomy have been likened to “lighting the fuse” for what is to follow (Tsao et al. 1999). Within minutes of injury, the axons in the tips of the proximal and distal stumps degenerate: the process is mediated by an intra-axonal rise in Ca^{2+} and activation of calpain (a Ca^{2+} -dependent serine-threonine protease). It has been suggested that this exquisitely localized acute axonal degeneration (AAD) creates a space for the numerous axon sprouts that emerge from the last nodes of Ranvier and the ends of the transected axons in the proximal stump, and/or enables an initial wave of Schwann cell proliferation to support the outgrowing sprouts (Wang et al. 2012). Since the ends of transected axons are rapidly sealed by Ca^{2+} -dependent fusion of vesicles, it is probable that the increase in Ca^{2+} in the distal axons is a combination of channel-mediated influx of extracellular Ca^{2+} and release of intracellular Ca^{2+} from axonal organelles such as mitochondria and endoplasmic reticulum. More distally, in a period of structural quiescence, axons appear morphologically intact and remain electrically excitable for 12–24 h, belying the fact that “the fuse is burning.” This latent period ends 24–48 h after axotomy when enzymatic digestion of axonal cytoskeletons spreads rapidly throughout the entire distal portion of the damaged nerve.

Acutely denervated Schwann cells dedifferentiate and divide. They respond to injury in the same way as injured neurons, by upregulating RAGs that collectively facilitate axonal regrowth (neurotrophins and their receptors and adhesion molecules) and downregulating expression of genes associated with the specialized activities of initiating, synthesizing, and maintaining myelin sheaths (e.g., P0, myelin basic protein, myelin-associated glycoprotein, PMP 22, periaxin, and Krox-20). Loss of axonal signaling promotes a switch in the Schwann cell phenotype



Fig. 1 (continued) undamaged axon. (f–k) events occurring within myelinated axons after transection and subsequent retraction of proximal and distal stumps. (f) three normal myelinated axons; (g, h) within a week of transection, axon sprouts and co-migrating Schwann cells grow into the inter-stump gap. Wallerian degeneration occurs throughout the distal stump; (i) axon sprouts and co-migrating Schwann cells cross the inter-stump gap and the axons enter Schwann tubes in the distal stump. The possibility that axonal misrouting will occur at this point is almost inevitable; (j, k) axon sprouts that do not cross the inter-stump gap may form a neuroma associated with the proximal stump. Almost all chronically denervated Schwann cells in the distal stump ultimately disappear. (For clarity, the responses of local connective tissues and blood vessels to injury have been omitted)

by triggering a rapid upregulation of the transcription factor c-Jun (Parkinson et al. 2004). The dedifferentiated “repair” phenotype resembles, but is apparently subtly distinct from, the immature Schwann phenotype (Arthur-Farraj et al. 2012). Increased expression of c-Jun probably triggers myelin breakdown by inhibiting Krox-20, in which role it may also act synergistically with Sox-2 (Decker et al. 2006; Parkinson et al. 2008; Salzer 2008). The sheaths are progressively fragmented by selective autophagy (myelinophagy) in a process that is positively regulated by the Schwann cell JNK/c-Jun pathway (Gomez-Sanchez et al. 2015). Myelin degradation is an essential component of the injury response because it removes components of the myelin sheaths such as myelin associated glycoprotein, MAG, that would otherwise inhibit axonal regrowth. The debris is degraded and cleared from the distal stump by two mechanistically distinct processes: a lectin-mediated, antibody-independent process mediated by Schwann cells and an antibody-dependent process mediated by endogenous and recruited hematogenous macrophages (Hirata and Kawabuchi 2002; Vargas et al. 2010). Recruited macrophages access the endoneurium via a focally leaky blood-nerve barrier, enticed by locally generated, Schwann cell-derived chemokines. These cells not only clear debris but also secrete a variety of chemokines, cytokines, and neuroactive factors, some of which may be associated with the generation and persistence of neuropathic pain (Chen et al. 2015). (For a review of the regulation of the cytokine/ chemokine network in peripheral nerve regeneration, see Dubový et al. 2013). One of the most obvious histological features of Wallerian degeneration is an increased number of Schwann cells within a recently denervated distal stump. Gliosis is driven by mitogens derived from axolemmal debris, macrophage-processed myelin, and fragments of fibronectin exposed by the action of Schwann cell-derived MMP-3. The newly generated Schwann cells populate the basal lamina tubes secreted by their parent cells, forming what are now usually called Schwann tubes, but which were previously known as bands of Büngner. They remain responsive to signals from regenerating axons for several months after injury. Axonal contact triggers a second wave of gliosis. Schwann cells contacted by proximally myelinated axons divide and differentiate into myelinating Schwann cells in a NRG1-dependent fashion (Glenn and Talbot 2013; Stassart et al. 2013): the new myelin sheaths are thinner and the internodal lengths (effectively Schwann cell territories) remain consistently shorter than in normal, uninjured nerve at ~300 μm (Hiscoe 1947; Vizoso and Young 1948; Ikeda and Oka 2012). Schwann cells in tubes that are not reinnervated ultimately become senescent and unresponsive to axonal signals (Li et al. 1997; Hall 1999; Sulaiman and Gordon 2000; Saheb-Al-Zamani et al. 2013).

Depending upon the type of injury and degree of surgical intervention, regenerating axons may extend either within the Schwann tubes that enclosed their parent axons; or within different tubes in either a coapted distal stump or an auto- or allograft; or within a non-neural environment such as the lumen of a nerve guidance conduit: they will not grow retrogradely within a proximal stump. If the basal laminae of the Schwann tubes remain intact, axonal growth cones will encounter the progeny of the original Schwann cells: the possibility of path finding

failures under these circumstances is low, and original targets will likely be reinnervated if the injury is “near-target.” In marked contrast, after tension-free coaptation of nerve stumps or insertion of a graft, no matter how meticulous the microsurgery, axonal growth cones will inevitably encounter unfamiliar Schwann tubes (de Medinacelli and Seaber 1989): the resulting axonal misrouting and nonspecific innervation of end organs compromises recovery, particularly of complex functions.

Paradoxically, because a transected axon gives off numerous collateral sprouts, the number of axons counted in a distal stump initially exceeds the number of axons in the corresponding proximal stump: the numbers may not fall for many months (Mackinnon et al. 1991). The presence of collaterals, most of which will ultimately be pruned, disguises the fact that axonal outgrowth is staggered over a period of several weeks. The earliest axon sprouts emerging from a proximal stump enter Schwann tubes apparently randomly, irrespective of their original functional modality, whereas axons that cross the suture line several weeks later are significantly more likely to reinnervate modality-appropriate tubes, a process Brushart originally termed “preferential motor reinnervation” (Brushart 1988). The timing of this switch from serendipitous to apparently preferential reinnervation of Schwann tubes may be enabled by upregulation of different neurotrophic factors in acutely denervated “motor” or “sensory” Schwann cells (Brushart 1993; Höke et al. 2006; Gordon 2014); other factors, such as differences in endoneurial microarchitecture, may also play a role in selective reinnervation (Madison et al. 2007; Kawamura et al. 2004).

Axons and co-migrating Schwann cells grow out from a proximal nerve stump into non-neural tissue in “minifascicles,” each surrounded by a very thin perineurial sheath: the phenomenon probably contributes to axonal dispersion across an empty inter-stump gap. Axons will not grow out from a proximal stump in the absence of ensheathing Schwann cells. Minifascicles that grow abortively into the epineurium and/or within the layers of the perineurium may produce painful, mechanosensitive neuromas around a lesion site.

Axonal regrowth is frustrated over time by progressive endoneurial fibrosis and Schwann cell senescence. Changes in fiber type and loss of striated muscle mass begin within days after denervation; up to 80% of muscle volume may be lost by 4 months and irreversible muscle fibrosis and fatty infiltration occurs after 2 years. Some reconstructive surgeons regard functional reinnervation as unlikely beyond 12–18 months, and even after “successful” repair, muscles usually exhibit weakness, impaired coordination, and reduced stamina; regeneration of the largest diameter axons and coactivation of α and γ efferents may fail. Cutaneous sensory receptors undergo a slow degenerative change after denervation and may disappear after 3 years. Their reinnervation tends to reverse these changes, particularly if the injury occurs close to the end organs and the nerves involved are sensory; however, the longer the period of denervation, the less complete will be the regeneration. Attempts to increase the rate of axonal regrowth in various experimental animal models remain disappointing.

2 The History of Nerve Repair

...“before the 19th century, surgeons worked without an inkling of nerve biology...” (Friedman 2009)

In civilian life, peripheral nerves are most likely to be damaged in road traffic accidents, penetrating trauma (both accidental and criminal), falls, and industrial accidents; they may also be cut, intentionally or inadvertently, during surgical interventions or disrupted during complicated obstetric deliveries. In modern warfare, peripheral nerves are frequently injured in blast and ballistic injuries. The history of nerve repair has been likened to the path of a yo-yo on an escalator (Walters 2015): it is difficult to disagree with that sentiment.

One of the oldest known medical texts is an Egyptian medical papyrus, bought in 1862 by an American archaeologist and known thereafter as the Edwin Smith papyrus. The roll of papyrus dates from around 1600 BCE but the hieratic script is thought to be copied from a document written some 1000 years earlier. The papyrus contains anatomical and clinical observations, diagnoses, treatment plans, and prognoses for 48 cases and deals mostly with wounds and trauma. Then, as now, not all cases were considered to be treatable. Although unaware of the pathogenetic mechanisms, the writer recognized that traumatic injuries of the spine could produce symptoms such as loss of sensation, paraplegia, weakness, priapism, and urinary incontinence, and that there was no treatment for a patient . . . “*who has a dislocation in a vertebra of his neck [and] who is unaware of his legs and his arms. . .*” (case 31). Nerve repair is not mentioned (Belen et al. 2009).

Human cadaveric dissection may have taken place in Babylon and in the Achaemenian dynasty (558 or 559–330 BCE) (Shoja and Tubbs 2007), but the weight of evidence suggests that the practice was usually forbidden in the ancient world because it violated all culturally acceptable and legal boundaries. Information about internal anatomy in the living would most likely have been obtained serendipitously, for example, during the treatment of gaping wounds, which means that almost all “known” human anatomy was extrapolated from animal dissection and vivisection. Medical schools flourished in Croton, Kos, Cnidus, and Alexandria. Teachers and students who worked in these schools included Alcmaeon (the “father of anatomy” (Chalcidius 1876); Hippocrates (the “father of rational medicine” and the forefather of neurology); Herophilus (also hailed as a “father of anatomy” or the “Vesalius of antiquity” [Wiltse and Pait 1998; Bay and Bay 2010]); Erasistratus; Galen; and Aretaeus.

Herophilus of Chalcedon (325–255 BCE) and Erasistratus of Chios (310–250 BCE) both worked in the great medical school in Alexandria during a brief period when human cadaveric dissection was permitted. Herophilus recognized that thread-like structures, *neura*, originated in the brain and spinal cord and not in the heart, contrary to Aristotelean cardiocentric views, . . . *the neura that make voluntary motion possible have their origin in the cerebrum (enkephalos) and spinal marrow* (Rufus Ephesius, *De anatomica partium hominis*, see Pearce 2008).

Erasistratus described sensory nerves as soft or hollow, initially believing that they were derived from the dura mater, while the motor nerves were hard or solid and derived from the brain and cerebellum. He subsequently traced all nerves to the brain, which appeared to him to be the origin of the bodily functions (Dobson 1927). Some 400 years later, in bloody vivisections using pigs that were often undertaken in public, Claudius Galen (129–201 CE) a Greek surgeon whose writing influenced medical thought for over 1500 years, demonstrated not only the muscles and nerves that mediate phonation by ligating and then releasing the recurrent laryngeal nerve but also that lesions of the spinal cord produced loss of movement and sensation below the level of the cut. (For further reading on Galen's experiments on the nervous system, see Wickens 2015).

Surgeons were reluctant to suture lacerated nerves. Contemporary authorities such as Hippocrates (ca. 460–370 BCE) and Galen adopted a nihilistic attitude, believing that nerves could not reunite and that manipulation of a severed nerve could lead to unbearable pain, spasms, and a cruel death. Their recommended treatments were heat (Hippocrates) or ground earthworms (Galen) (Friedman 2009). That said, a recent translation of *Alâim-i Cerrâhîn* (Wonders of Surgeons) contains a tantalizing anecdotal report that Hippocrates successfully “sutured” a cut nerve at the ankle using a woman's hair (Belen et al. 2009).

After the fall of the Western Roman Empire in the fifth century CE, scholarship and learning in Western Europe were dominated by the stultifying, infallible authority of the Christian Church and the scientific enquiry that had so energized Greek physicians and scholars in previous centuries stagnated for the next thousand years. Surviving manuscripts and books, including a substantial number of Galen's many works, were translated into Farsi, Syriac, and Arabic and then copied and distributed in Southern Italy, Byzantium, and throughout the Islamic world during the Islamic Golden Age (Middle Age) of medicine (approximately seventh to thirteenth century CE) (Abdel-Halim 2001). Persian anatomists and physicians upheld some of the misconceptions of their ancient Greek sources, refuted others, and added their own interpretations (Green 2003). The translated writings of Paulus Aeginatus (approximately 626–696 CE), a celebrated surgeon of the early Byzantine era, significantly influenced the clinical practice of a number of surgeons of the Islamic schools. Paulus generally demurred from using sutures to repair injured nerves, preferring instead to apply unspecified “agglutinatives” to bring stumps together. Rhazes (850–932), Avicenna (980–1037 CE), and Albucasis (936–1013) also acknowledged that the ends of injured nerves should be approximated, possibly using sutures (Terzis et al. 1997; Goodrich and Kliot 2015). Rhazes recognized that outcomes varied according to the mechanism of injury, teaching his students that . . . “*nerve function is abolished when the nerve is totally sectioned from a contusion, or compression, oedema or tumour or from a severe cold that affects it. However, nerve lesions from oedema, compression or cold may be reversed when the cause is treated. . . if there is section of the nerve there is no therapy*” (Souayah and Greenstein 2005).

By the end of the twelfth century, renewed interest in the natural sciences was spreading slowly across Western Europe. Arabic translations of the ancient Greek

scientific texts were translated into medieval Latin, particularly in the Toledo School of Translators in the twelfth and thirteenth centuries (Arraez-Aybar et al. 2015), and circulated widely. The curricula of the first major centers of medical teaching in Europe (in Salerno, Bologna, Montpellier and Paris) were based on translations of Galen and Avicenna: their authority ensured that these works remained required reading until at least the seventeenth century in many medical schools (Moosavi 2009).

During the thirteenth and fourteenth centuries, a number of influential European surgeons, including Roger Frugard (1140–1195), Guglielmo da Saliceto (1210–1280), Guido Lanfranchi (1250–1306), and Guy de Chauliac (1300–1368), alluded to the practice of suturing transected nerves in their writing, although none described their technique in detail. Roger Frugard (often misleadingly referred to as Roger of Salerno) was probably the first “modern” western writer on surgery (Hunt 1994) and a pioneer in the treatment of nerve damage. He advocated re-anastomosis of nerve stumps, cautioning the surgeon to pay particular attention to the re-alignment of the nerve ends. The first chapter of *Chirurgia Parva* by Guido Lanfranchi (often called Lanfranco of Milan or Lanfranc) contains a section entitled *De incisione neruorum* (about cut nerves) in which he advises sewing the ends of the nerve together with the skin. Like Galen had done many centuries earlier, Lanfranc recommended the value of boiled earthworms (*wormys of the erthe*) as a treatment (Simpson 2009) while also cautioning that . . . “*a drawynge togedre of a synwe . . . is a cause of a crampe . . .*” (von Fleischhacker 1894). The use of the word “*synwe*” (sinew) in this translation of Lanfranc’s text is a reminder that from the days of the ancient Greek scholars, physicians and anatomists not only struggled conceptually to differentiate between nerves, tendons, sinews, and ligaments, whether in their patients or in cadavers, but also semantically: as late as the seventeenth century, the words *nervus* (Latin) and *sinew* or *synwe* (English) were often used interchangeably in Latin and English medical texts (Simpson 2009). De Chauliac advised debridement of a wound to avoid the intervention of non-nervous tissue between the apposed nerve stumps: his book *Chirurgia Magna* remained a standard surgical text for some 300 years.

Gabriele Ferrara (1543–1627) is generally credited with providing the first detailed technical description of nerve suture in *Observationum Chirurgicarum: Observatio XVII: De Modo Consuendi Nervos Magnos Incisos*. The needle and suture thread of split tortoise tendon were to be washed in a decoction of red wine, rosemary, and roses, which, although unappreciated at that time, would have provided some alcoholic disinfection of the instruments. The nerve ends were to be sutured gently, under minimal traction, taking care not to damage the retracted stumps further by using too many sutures. A hot mixture of hypericum oil and spruce oil was applied to insulate the sutured segment, after which the limb was splinted and the patient confined to bed to immobilize the limb and avoid damaging the suture (Artico et al. 1996). Minimizing tension at the suture site and isolating the repair from the wound bed (although not with hot oil) inform current surgical practice.

Descriptive, topographical anatomy based upon cadaveric dissection enjoyed a golden age of discovery in the sixteenth century, much of it centered on the medical school in Padua. In 1543, the same year that *De revolutionibus orbium coelestium*

was published by Nicolaus Copernicus, Andreas Vesalius, a Belgian anatomist and surgeon working in Padua, published *De Humani Corporis Fabrica Libri Septem*: the books changed the view of the internal anatomy of the body. Based on knowledge gained from his own meticulous cadaveric dissections, Vesalius demonstrated that the human body, not ancient texts, was the only reliable source of accurate anatomical information, challenging Galen's long-standing anatomical authority (Standing 2016). In writing about the possible function of nerves, Vesalius was less iconoclastic, retaining a mechanistic view of nerves as conduits that persisted in some guise or other until the nineteenth century . . . *"Nerves therefore serve the same purpose to the brain that the great artery does to the heart, and the vena cava to the liver, in as much as they convey to the instruments to which it ought to be sent the spirit prepared by the brain, and hence may be regarded as the busy attendants and messengers of the brain"* (Ford 1901).

Most anatomists at this time were also surgeons, . . . *"men who would push forwards the frontiers of surgery as far as possible with the primitive means available to them. . . and with primitive ideas of the body's physiology and of the underlying pathology of most of the diseases they encountered"* (Ellis 2009). The list of conditions that a surgeon might treat armed with these "primitive ideas" included fractures, vascular injuries, hernias and bladder stones. As for repairing nerve injury surgically . . . *"the pendulum swung back and forth between treatment and no treatment for centuries. . ."* (Walters 2015). Surgeons remained reluctant to operate on the grounds that repair were commonly followed by severe postoperative pain that could only be relieved by secondary, elective transection of the "repaired" nerve. This concern is evident in a letter probably written in 1585 from Etienne Gourmelen (Dean of the Faculté de Médecine at the Collège de France) to Ambroise Paré, about the danger of iatrogenic nerve injury when ligating vessels. Note that Gourmelen acknowledges Galen's persisting influence on medical practice . . . *"if with the needle one should prick some nervous part, to wit even the nerve itself, when he wishes. . . , grossly to constrain the vein in tying it, necessarily there will follow a new inflammation, from the inflammation a convulsion, from the convulsion, death: for fear of which accidents Galen never dared to stitch transverse wounds (that which is always less dangerous) before uncovering the aponeuroses [sic] of the muscles. . ."* (Hernigou 2013). Ambroise Paré, an army surgeon, recognized that nerves could be injured by . . . *"the violent incursion of externall things. . . which contuse, batter and grinde in sunder, as by the blow of a stone, cudgel, hammer, lance, bullet out of a gun or crossbow; by the biting of greater teeth; or the pricking of some sharpe thing . . . or the edge of some cutting things, as a sword or rapier; or of stretching things which violently tear asunder the nervous bodies". . .* (1634 English translation of Paré, cited in Holmes 1951), but could offer little remedy other than treating the accompanying pain.

Peripheral nerve does not appear to have been a popular tissue for early light microscopists to examine, doubtless because in its unfixed state it is not particularly easy to handle or to interpret, as will become apparent later. Fortunately, some were not deterred. In 1675 Anton van Leeuwenhoek described the structure of slices of dried optic nerve, using lenses that he had ground and that were capable of achieving magnifications of over 200 $\times$, . . . *"I have put before my microscope a piece of such a*

dried Optic Nerve of a Cow...in all the places that are left white and clear are cavities in the dried Nerve which I imagine have been filaments and out of which for the most part soft globules have been exhaled..." Some 2 years later, Leeuwenhoek commented that the optic nerves contained ...*"diverse very small threads or vessels lying by each other."* His observations caused him to doubt that nerves were hollow tubes, a view that had prevailed since the ancient Greek belief that the tubes conveyed "animal spirits"...*"I took eight different optic nerves which did shrink upon which a little pit comes to appear about the middle of the nerve and it is this pit in all probability that Galen mistook for a cavity"...*(Ford 1981, 1982). Despite van Leeuwenhoek's lucid descriptions and meticulous drawings (see Boullerne 2016), there was a popular consensus that nerve fibers were composed of globules. As Ranvier pointed out two centuries later, not only was van Leeuwenhoek uniquely skilful, but this erroneous view of what a nerve fiber looked like was entirely of the anatomists' own making: ...*"L'anatomie des nerfs faisait donc avec Leeuwenhoek un grand progrès; mais comme il observait à l'aide d'appareils d'optique qu'il construisait lui-même avec une habileté consommée, il était le seul en Europe à cette époque qui en possédât de suffisants pour reconnaître des détails aussi fins; c'est pour cela que l'exactitude de ses découvertes n'a été constatée que bien longtemps après lui. En effet, la plupart des anatomistes du siècle dernier et du commencement de ce siècle ont eu de tout autres idées sur la constitution des nerfs. Comme ils les dissociaient dans l'eau, les recouvraient d'une lamelle épaisse et exerçaient sans doute une assez forte pression, ils voyaient sous le microscope une quantité de granules ou de globules, et ils supposaient que la substance médullaire du nerf en était formée..."* (Ranvier, Deuxième Leçon, 7 Décembre 1876).

Some 60 years after the publication of the paradigm shifting *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus* by William Harvey (1628) that had established the way in which blood circulated around the body, ideas about nerve function still relied upon ancient mechanistic theories. Giovanni Alfonso Borelli (1681) (the father of spinal biomechanics, Provencher, and Abdu 2000) described nerve fibers as being neither ...*"solid, full and impermeable, nor are they tubes hollow and empty like reeds, but ... canals filled with a certain spongy material like elder-pith. Such a marrow of the fibres can easily be moistened by the spirituous juice of the brain, to which it is conjoined, and may indeed be saturated to turgescence, as we see sponges are saturated by water in contact with them."* Borelli believed that the unspecified fluid inside a nerve transmitted "commotions" from the brain along the nerve to the muscle it innervated, at which point drops of fluid were ejected into the muscle, causing it to contract (Foster 1901). A century later, the work of Luigi Galvani (1780) laid the foundations of the concept that nervous activity involved electricity rather than the physical movement of a fluid (Schuetze 1983).

... "Almost everyone who has published anything upon the subject differs to some degree at least from other writers..." (Howell and Huber 1892)

William Cumberland Cruikshank (1795) is credited with the first experimental studies to explore peripheral nerve regrowth. He removed a 15 mm section of

nerve from the cervical vagus nerve of a dog and the dog survived; when he removed a section of the contralateral cervical vagus 8 days later, the dog died. On visual examination of both nerves *post mortem*, he found that the interstump gaps appeared to be filled with a substance . . . “*of the same colour as nerve, but not fibrous*” . . . , which he took as evidence of new nerve growth. He repeated the experiment but waited 3 weeks before cutting the contralateral nerve and the dog survived (Cruikshank and Hunter 1795). For reasons beyond Cruikshank’s control, his findings were not published until 1795, just before John Haighton published a paper on regeneration of the canine vagus nerve (Holmes 1951; Ochs 1977). Haighton was perhaps one of the first to recognize that the response of a peripheral nerve to injury was unlike that of other tissues: . . . “*There are few who will deny that a bone, when fractured, fills up the chasm with a substance of its own kind; or that a tendon, when divided, repairs with a substance resembling itself. But this law of nature is not admitted as universal; and this power of repairing in kind has been denied to several of the constituent parts of the animal machine With respect to nerves, it has been both affirmed and denied: some assert that the new formed substance possesses the characters of the primitive nerve; others maintain that it is totally different; and both have their opinions on experiment reported. . .*” Haighton added a crucial step to Cruikshank’s experimental model in that he delayed cutting the contralateral vagus for 6 weeks. The dog recovered, becoming a yard dog for 19 months and gradually regaining its bark as its overall health improved; however, it died when both vagi were transected. Haighton concluded that divided nerves were capable of reuniting and that . . . “*the new formed substance is really and truly nerve.*” He anticipated today’s requirements that novel therapeutic protocols for nerve repair should be evaluated using histological, molecular, functional, and behavioral analyses as follows. “. . . *I am persuaded that anatomy can determine only the presence and existence of an [sic] uniting medium; but it is the province of physiology to decide whether the medium of union possess the characters, and perform the function, of the original nerve. . .*” (Haighton 1795).

Determining the nature and source of the material that plugged the gap between the two ends of a transected nerve was highly controversial – was it an outgrowth from the central stump or a reunion of the central and peripheral stumps, i.e., did healing occur by “first intention”? These questions, posed at a time when the normal structure and function of peripheral nerves were still matters of speculation, reverberated until the definitive formulation of the neuron doctrine in the last decade of the nineteenth century (Waldeyer-Hartz 1891; Cajal 1954, 1991; Mazzarello 1999; Glickstein 2006; Guillery 2007). The debate had been driven by very different concepts of the relationship between a nerve cell body and a nerve fiber (axis cylinder, axon). It was fuelled by the often contradictory findings of clinical data; the misinterpretation of outcomes, such as the dismissal of experiments as “unsuccessful” because too little time was allowed for functional recovery, even though Swan (1820) and Prévost (1826) had demonstrated that regeneration took months rather than days; and the inability to visualize regrowing axons in experimental studies until the introduction of the reduced silver nitrate technique revealed neuronal individuality (Remak 1838; Steinrück 1838; Nasse 1839; Günther and Schön

1840; Waller 1850, 1852; Bruch 1855; Philipeaux and Vulpian 1859, 1860; Ranvier 1878). Polygenists believed that nerve cell bodies and nerve fibers were entirely independent structures and that axons regenerated autogenously in a peripheral stump either *de novo* from viable (but changed) axons or from chains of coalescing Schwann cells, and then fused with the axons in the central stump (the reunion or catenary concept), (Lat. *catena*, chain) (e.g., Vulpian 1866). The idea, though ultimately shown to be wrong, was not unreasonable, given that it was known that repair of damaged tissues such as bone was a local process. Monogenists such as Waller (1850, 1852) and Ranvier (1878) believed that nerve fibers were continuous with, and derived trophic support from, their centrally placed nerve cell bodies, and that new axons sprouted from the cut fibers in the central stump and invaded the denervated peripheral stump (outgrowth concept). Their comments prefigure twentieth-century concepts of axoplasmic transport: ...*“A nerve-cell would be to its efferent nerve what a fountain is to a rivulet which trickles from it – a centre of nutritive energy”* (Waller, in Sykes 2000); ...*“Lorsqu’un nerf a été coupé, la régulation de la nutrition étant supprimée dans la partie qui est séparée du système nerveux central. . .”* (Ranvier 1878).

Augustus Waller summarized the degeneration that would come to bear his name in a report read to the Royal Society of London on February 21, Waller 1850. He described the gradual disintegration of the glossopharyngeal or hypoglossal nerves of frogs after axotomy (Koeppen 2004). ...*“During the first two or three days after section [of a glossopharyngeal nerve], no alteration in the texture and transparency of the tubes of the papillary nerves can be detected. Generally, at the end of the third and fourth day, we detect the first alteration by a slightly turbid or coagulated appearance of the medulla, which no longer appears completely to fill the tubular membrane, which does not appear to be affected. . . . About five or six days after section, the alteration of the nerve-tubes . . . has become much more distinct, by a kind of coagulation or curdling of the medulla into separate particles of various sizes. . . . About the twentieth day the medullary particles are completely reduced to a granular state.”* The significance of Waller’s work was later acknowledged by Ranvier (1876): *“Waller . . . put arriver à formuler cette loi générale de la dégénération: un tube nerveux dégénère lorsqu’il est séparé de son centre trophique. La découverte des centres trophiques a été une des plus importantes pour l’histologie et pour la physiologie du système nerveux.”*

The spotlight of attention is focused upon this problem during every war. . . . (Horn 1946)

Given the destructive powers that are unleashed during military conflicts, it is not surprising that peripheral nerve injuries are more common in wartime than in peacetime. For example, in the first year of WWI ...*“more than 1500 casualties with [peripheral nerve injuries] swamped the Val de Grâce Military Hospital in Paris. . .”* (Hanigan 2010). Over the centuries, wars have provided tens of thousands of opportunities for surgeons to develop their skills in diagnosing and managing such injuries. However, until the early part of the nineteenth century, military surgeons adopted a conservative approach and rarely intervened specifically to repair

a damaged nerve. In discussing John Haighton's findings of regeneration of the canine vagus nerve in the context of his own experience of treating soldiers who had sustained severe injuries on the battlefield (for whom he could offer little alternative to excision of the affected part of the nerve or amputation of a painful limb or digit), G J Guthrie wrote that he knew of no case in man of recovery of function after division of a main nerve and concluded that . . . "*there is an essential difference in the powers of reproduction in nerves between the lower animals and man; and that experiments made on the former . . . ought to be received with considerable caution. . .*" (Guthrie 1827).

In May 1863, during the American Civil War, Dr. William Hammond, then Surgeon-General of the US Army, ordered that certain wards should be set apart for the treatment of diseases of the nervous system in the US Army Hospital in Christian Street, Philadelphia. He asked Drs Silas Weir Mitchell and George Morehouse to share the medical charge of these wards. Weir Mitchell subsequently published reports of the experience in two books. *Gunshot Wounds and Other Injuries of Nerves*, written with Morehouse and Dr. William Keen in 1864, is regarded as the first organized study of nerve injuries and is perhaps best remembered for its detailed descriptions of causalgia, "*a condition that will always remain associated with the name of Weir Mitchell*" (Seddon 1942; Birch 2009). The authors summarize the unsatisfactory contemporary management of nerve injuries . . . "*in the great monographs of military surgery, this defect [in treating nerve injuries] is so complete that wounds of nerves are there related rather as curiosities and matters for despair, than with any view to their full clinical study and systematic treatment.*" In the second book, *Injuries of Nerves and their Consequences*, published in 1872, Weir Mitchell refers frequently to Waller's "admirable work," recommending it to all those who treat nerve injuries . . . "*since a clear knowledge of his conclusions lies at the basis of the subject of nerve changes after section.*"

It is hard to decide where history stops and contemporary development of peripheral nerve surgery begins. (Little et al. 2004)

Waller's observations were confirmed and extended by others, including Aldo Perroncito working in Golgi's laboratory (Perroncito 1905) and a Romanian group led by Georges Marinesco. Probably the work that is best remembered is Santiago Ramón y Cajal's seminal description of degeneration and regeneration as assessed light microscopically, published in *Estudios Sobre la Regeneración del Sistema Nervioso, Volume 1* (1913) and later hailed as . . . "*one of the most extensive studies of the histological phenomena associated with degeneration and regeneration of peripheral nerves ever published*" (Jones 1999). That said, most of what is known about the structure and function of healthy and diseased peripheral nerves is a product of the technical and methodological advances in microscopy, immunohistochemistry, tissue culture, genomics, proteomics and electrophysiology that drive modern bioscientific research. An extensive literature documents aspects of peripheral nerve biology such as the normal development and maintenance of axon-Schwann cell relationships, the structure of the myelin sheath, the ionic basis of

action potential propagation, the role(s) of the components of the endoneurial extracellular matrix, and the major cellular and molecular events triggered by nerve injury (Fig. 1). As will become apparent, some of that research has translated from lab bench to clinic.

Although physicians have long observed that functional outcomes after nerve injury varied according to the type of injury, a formalized codification of their empirical correlations is relatively recent. In the 1940s, Sir Herbert Seddon added the terms neurapraxia, axonotmesis, and neurotmesis to the clinico-pathological lexicon out of a concern to categorize... *“those numerous cases in which function is lost although anatomical continuity of the nerve is more or less preserved,”* commenting that the... *“three types of lesion have been produced experimentally, and work of this kind has contributed considerably to our understanding of them”* (Seddon 1942). (Strictly speaking, it should be noted that Seddon attributed the derivation of the three level classificatory scheme to Sir Henry Cohen). Sir Sydney Sunderland subsequently extended Seddon's triplet, distinguishing between progressively more invasive levels of injury to the endoneurium, perineurium, and epineurium, respectively, and correlating these new grades of axonotmesis with more accurate prognoses of outcomes (Sunderland 1951) (Table 1). Mackinnon and Dellon added a Grade 6 “mixed” injury to Sunderland's classification, to better reflect the common clinical scenario of a mixture of crush and transection injury occurring within a damaged nerve (Mackinnon and Dellon 1988). Seddon had previously reflected the common clinical perception that... *“by no means all nerve injuries can be classified under simple headings. . . . An injury may affect the fibers of a nerve in varying degree, and the clinical picture will then be more complex. A lesion may be made up by a combination of any of the following: (a) neurotmesis; (b) axonotmesis; (c) neurapraxia; (d) normal fibers”* (Seddon 1942). Recovery is typically complete after Sunderland's grade 1 (equivalent to neurapraxia) and grade 2 (axonal degeneration with intact endoneurium) injuries; grade 3 injuries recover partially; grades 4 and 5 (equivalent to neurotmesis) and grade 6 “mixed” injuries almost inevitably require surgical intervention.

Dr. Arnemann of Göttingen is credited with performing the first “modern” suturing of an experimentally divided nerve in 1787 (Holmes 1951). Being unaware that axonal regrowth is a lengthy process, he abandoned the experiment too soon, before obtaining any evidence of functional recovery. Perhaps it was this unsuccessful experience that caused him to “deny positively” some of Haighton's experiments and to declare that true nervous substance is never reproduced (Haighton 1795). The practice of suturing the ends of cut nerves together was used largely as an experimental tool for the greater part of the nineteenth century. The occasional clinical reports of neurorrhaphy revealed variable outcomes. In 1836, Baudens sutured the cut ends of four nerve trunks in the upper arm after they had been transected by a sabre cut during a duel: the patient died 8 days later and at post-mortem the nerves were found to have separated. In 1854, von Langenbeck reported the first successful repair of a median nerve laceration, apparently with complete return of function 1 year later. It is reasonable to assume that the increasing use of anesthesia during the nineteenth century rendered nerve suture a less harrowing procedure for both patient

Table 1 Classification of nerve injuries and associated pathological changes

Seddon	Sunderland	Mackinnon and Dellon	Pathology
Neurapraxia	Grade 1		Occurs when compression or stretch produces an anoxic, physiological block of both axoplasmic transport and ion channel functions along affected axons. Axonal continuity is retained: Loss of function is therefore temporary and release of the compressive agent usually results in rapid and complete recovery of function and relief of pain (3–6 weeks). A more substantial and prolonged mechanical compression or stretch is most likely to cause a focal demyelination that may be paranodal or affect whole internodes
Axonotmesis	Grade 2		Occurs when a blunt injury to a nerve severs axons but the overall continuity of the nerve fiber is intact. Axons degenerate distal to the site of the lesion, irrespective of caliber or functional modality. Conduction ceases throughout the distal extent of the affected part of the nerve within a few days of injury. Schwann cell basal lamina tubes either remain continuous across the lesion or are minimally separated within a morphologically intact perineurium and epineurium. While there is no <i>a priori</i> reason, why regrowing axon sprouts should not extend to their appropriate targets within the Schwann cell tubes that housed their parent axons, the distance from injury site to end organ(s) may ultimately defeat successful reinnervation. If regrowing axons are not impeded in their travel, recovery progresses proximo-distally (as judged by an advancing Tinel's sign) at a rate of ~1 mm/day. However, even when an axon reaches its target organ, reinnervation of that target is neither guaranteed nor is it synonymous with full functional recovery
Axonotmesis	Grade 3		Same as for grade 2, axons degenerate distal to the site of the lesion, but endoneurial integrity is disrupted
Axonotmesis	Grade 4		Same as for grade 2, axons degenerate distal to the site of the lesion, but perineurial integrity is disrupted
Neurotmesis	Grade 5		Occurs when a nerve is either completely divided or so badly disorganized by injury that recovery without some form of surgical intervention is impossible. All affected axons in the extreme tip of the proximal stump and throughout the distal stump degenerate, irrespective of their caliber or functional modality: Some neurons may die, especially if the injury is close to their cell bodies. A wide inter-stump gap may be produced either

(continued)

Table 1 (continued)

Seddon	Sunderland	Mackinnon and Dellon	Pathology
			by the injury <i>per se</i> or during subsequent intraoperative wound debridement. Distance and time are significant factors in recovery, particularly after proximal injuries in limb nerves: Inevitably, many regrowing axons will be misdirected into modality-inappropriate Schwann tubes in the distal stump and/or will fail to reach their target organs before the latter disappear
		Grade 6	A mixed injury with varying degrees of injury in some fascicles and normal function in others

and surgeon and, together with a developing appreciation of asepsis, changed the indications for nerve repair. In a comprehensive review of 164 cases where nerve stumps were either “immediately” sutured (primary suture) or from a few days to years after the initial injury (secondary suture), Howell and Huber reported that “*The prognosis of cases of ‘primary nerve suture’ is very favourable; in all probability function will be restored either completely or partially. . . . The prognosis is more favourable the younger the patient. . . and ‘improvement is almost certain’... after secondary suture* (Howell and Huber 1893). (Refer to Walters (2015) for a detailed account of the various techniques of end-to-end suture repair of nerves as described by Woolsey (1907); Tinel (1917); Babcock (1927) and others in the early twentieth century).

In his dissertation on the treatment of “morbid local affections of nerves,” Joseph Swan had concluded that . . . “*When a portion of a nerve has been removed [in the sciatic nerves of dogs or rabbits], and especially if it be a large one, the breach is only with the greatest difficulty, if ever, repaired*” (Swan, 1820, cited in Holmes 1951). A peripheral nerve recoils approximately 10% of its length when it is transected (Topp 2015): this “retraction gap” contributes to the critical gap distance (Orf 1981). Nerve stumps separated by a short gap (<1 cm) may be sutured together (after adequate mobilization) providing there is no tension at the suture site. The inter-stump gap may be shortened mechanically, by either *in situ* mobilization, rerouting and transposition, joint positioning, or, very rarely, bone shortening, in order to bring the nerve stumps together without tension (Trumble and McCallister 2000; Jobe and Martinez 2013). Babcock produced an anatomical map of positions involving joint flexion designed to eliminate inter-stump gaps of up to 10 cm (Babcock 1927). Clinical outcomes were often disappointing, not least because . . . “*subsequent straightening [of the limb] tears apart the line of suture of the inelastic nerves and good function is almost unattainable*” (Dandy 1943). Experimental studies in dogs (Highet and Sanders 1943) and a review of clinical cases where no recovery had occurred after extensive postoperative stretching (Highet and Holmes 1943) revealed that the practice produced extensive axonal degeneration, vascular disruption, and extra- and intraneural fibrosis at suture sites. A recent experimental study in rats confirmed and extended these findings, showing

that high-tension coaptation impairs Schwann cell activation (Yi and Dahlin 2010). Current clinical practice limits elongation of a nerve to approximately 10% of its original length to avoid compromising intraneural perfusion (Jobe and Martinez 2012).

A long defect, produced either at the time of injury or following wound debridement or secondary revision, is usually bridged with a graft or some other form of axonal guide (Millesi 1986). Philipeaux and Vulpian, proponents of the autogenetic basis of nerve regeneration, are credited with the first experiment, grafting a segment of lingual nerve into the hypoglossal nerve of a dog in 1870 (Dellon and Dellon 1992). Almost a century later, reflecting on his clinical experience during WWI, Tinel recommended that where an inter-stump gap was too great to bring the ends together without tension at the suture site . . . “*the only legitimate operation is nerve grafting*” (Tinel 1917).

The compressive pressures exerted by adjacent tendons, muscles and bones during normal bodily movements and postures expose peripheral nerves to combinations of tensile, shear and compressive stresses that they are designed to accommodate. Normally, a nerve glides against its surrounding tissues as it adapts to length differences during limb movement (Millesi 1986). In marked contrast, postoperative fibrosis tethers a regenerating nerve within its wound bed, restricting or even preventing the excursions that should occur during movement. The longitudinal traction and compressive stress this imposes on the nerve compromises its local microcirculation and impedes axonal regeneration (Driscoll et al. 2002; Ju et al. 2006). Early attempts to protect a neurorrhaphy site with some kind of wrap (e.g., Cargile membrane) were counterproductive because they stimulated fibrosis . . . “*Isolation of the nerve by an aponeurotic flap, a muscular bed, a fatty covering, has been proposed; catgut has been rolled round the nerve; it has been enveloped in a peritoneal flap or a layer of amnion; attempts have even been made to wrap round it a thin sheet of aluminium or of rubber; the two united fragments have been brought into a segment of a vein or an artery; a few drops of gomenol [an oil obtained from *Melaleuca viridiflora*] have been injected around the nerve. . . . In our opinion, these practices are almost always useless, and even harmful in many cases, especially as regards the use of foreign bodies*” (Tinel 1917). Current practice indicates that Tinel’s disparagement of the use of autologous vein or amnion to enwrap a primary neurorrhaphy appears to have been misplaced (Henry et al. 2009; Leuzzi et al. 2014); biological or biodegradable barriers may facilitate recovery, particularly where there are associated vascular or tendon injuries (Sadek et al. 2014). An “off-the-shelf” wrap of processed porcine extracellular matrix has been developed for clinical use (Kokkalis et al. 2011).

In the problem of transplantation of nerves the question of the fate and survival and multiplication of the cells of Schwann is of importance. . . . (Ingebrigtsen 1915)

The current gold standard protocol to reconstruct a long gap (>3 cm) in a motor or mixed sensory/motor peripheral nerve is an autograft, typically based on superficial sensory nerves e.g., sural nerve, medial or lateral cutaneous nerves of the forearm, or

superficial or deep fibular nerves (but see comments by Birch on the use of the term “gold standard” in the context of nerve grafting (Birch 2011)). Autografts contribute all the cellular and acellular components of a peripheral nerve except neurons; somewhat counter-intuitively, their use does not guarantee a favorable outcome, particularly for mixed sensorimotor nerve function. Protocol-specific disadvantages include limited tissue volume (which may be significant when dealing with defects in large nerves in the limbs); fascicular and modality mismatches between host and donor nerves (all properties of the graft); secondary donor site morbidity such as formation of a symptomatic neuroma, potential infection, hyperesthesia, or numbness within the distribution of the donor nerve (all consequences of harvesting the graft); and increased operating time. Large caliber “trunk” grafts may be insufficiently vascularized from the recipient tissue bed, rendering them incapable of supporting axonal regrowth (Dellon 1992).

“The clinical problem associated with misdirection of regenerating nerves remains one of the most vexing issues of functional recovery in the patients who suffer injuries that result in degeneration of the disconnected distal nerve stump and require surgical repair” (Gordon 2014). The predominant use of sensory autografts in the clinic is predicated on the assumption that they will support regrowing motor (somatic and autonomic) axons as well as sensory axons. While these grafts clearly support axonal regrowth, do modality-inappropriate grafts adversely affect either the rate or the extent of axonal regrowth? Animal studies exploring the concept of modality-specific regeneration have produced conflicting results, likely influenced by different experimental models (Nichols et al. 2004; Mackinnon 2010; Neubauer et al. 2010). Apparently, preferential reinnervation of modality-appropriate Schwann tubes may be enabled by differing Schwann cell phenotypes that are retained after denervation (Brushart 1993; Höke et al. 2006; Gordon 2014) and/or by the differing internal architecture of motor and sensory nerves (Lloyd et al. 2007).

The alternatives to autografting are allografting, end-to-side (termino-lateral) neurorrhaphy, nerve transfer, and entubulation, all protocols that were mooted in the nineteenth century and all with a chequered history. Collectively they represent the area of peripheral nerve surgery where basic science and clinical science work most closely – what has been called “histological surgery.” Eduard Albert may have performed the first human nerve allograft in September 1876, when he reconstructed a post-excisional long gap in the median nerve of Franziska Regensburger using a segment of tibial nerve from a freshly amputated leg. The surgery was technically successful but the outcome was a failure from the patient’s perspective: sensation and motility were not restored (Schmidt 1993). In February 1889, Mayo-Robson read a paper before the Clinical Society of London . . . *“on a case of nerve grafting, which was I believe, the first in which such an operation had been performed in this country on the human subject.”* In an operation that was strikingly similar to that described by Albert, Mayo-Robson had (allo)grafted nerve from a freshly amputated limb into a post-excisional defect in the median nerve of a 14 year old girl, reporting that . . . *“recovery was perfect in every respect”*. . . at 3 year review (Mayo-Robson 1917).

The contemporary literature contains a number of descriptions of the use of 3 cm or 5 cm grafts of the sciatic nerves of kitten, rabbit, dog, and bullock and the spinal

cord of a rabbit to repair long defects in median, ulnar, or sciatic nerves, with “*widely varying results*,” but apparently some success (Ballance and Stewart 1901; Mayo-Robson 1917). It is difficult to interpret these successful results other than to assume that regenerating axons bypassed the allografts to innervate their targets, accounting for the successful outcomes reported at long-term review. Of greater significance, these reports speak to the importance clinicians afforded to reestablishing the continuity of a damaged nerve across a long defect.

Albert and Mayo-Robson would have been unaware of the need to mitigate their patients’ inevitable immune-mediated rejection of allo- and xeno-grafted nerve when they operated in the latter part of the nineteenth century. Experimental evidence that these types of transplants became necrotic and failed to support axonal regeneration appeared early in the twentieth century: “*Heteroplastic transplanted nerves become necrotic. They are unsuitable for bridges in cases of nerve defects, and my results explain the failure of the attempts at heteroplastic transplantation of nerves in human beings*” (Ingebrigtsen 1915). An increasing understanding of allograft rejection in transplanted tissues (Barker and Markmann 2013) and of the pathobiology of nerve injury underpinned a renewed interest in using cadaveric nerve allografts to repair long nerve defects later in the twentieth century (Bain et al. 1992; Fish et al. 1992; Evans et al. 1994). The attraction is obvious: peripheral nerve allografts offer access to size-, length- and modality-matched tissue, avoid donor site morbidity and reduce operating times. The downside is that cellular allografts are also immunogenic, which means that graft recipients require systemic immunosuppression, predisposing them to the risks of opportunistic infections, neoplasia, and toxicity-induced side effects.

Currently, patients who receive cellular allografts require systemic immunosuppression for approximately 24 months, by which time it is presumed that host Schwann cells have repopulated the graft. Removing the cellular content of a peripheral nerve allograft reduces its immunogenicity, bypassing the need for post-operative immunosuppression, while retaining those elements of the endoneurial extracellular matrix that are known to facilitate axonal regeneration, in particular, the laminin-rich basal lamina tubes that surrounded each axon-Schwann cell unit in the donor nerve. Techniques such as lyophilization, cold preservation, detergent processing, and irradiation all produce acellular allografts that are immunologically tolerated and that perform reasonably well in experimental small animal models. Their ability to support axonal regeneration varies, reflecting the degree of endoneurial disruption produced by different pretreatments. A decellularized Schwann cell basal lamina tube is necessary but not sufficient to support axonal regrowth: axons will not grow into basal lamina tubes in acellular peripheral nerve grafts unless they are accompanied by Schwann cells (Hall 1986a, b). The critical length of an acellular graft (the length beyond which the graft is unable to support axonal regrowth) appears to be determined by the gradual quiescence of the migrating non-axon-associated Schwann cells (Saheb-Al-Zamani et al. 2013), which means that the extent of axonal regeneration into acellular grafts without the addition of exogenous Schwann cells is limited to ≤ 3 cm (Moore et al. 2011; Brooks et al. 2012; Szyndrak et al. 2013; Rinker et al. 2017). Only one acellular, processed nerve allograft is available commercially, apparently prepared by treating human nerve

with detergent (to remove cellular debris), chondroitinase (to remove chondroitin sulphate proteoglycan, an axonal growth inhibitor, Graham et al. 2007), and γ -irradiation sterilization (Moore et al. 2011).

End-to-side neurorrhaphy, in which the distal end of an injured nerve is sutured to the side of an intact donor nerve, was first described by L  ti  vant in 1873, abandoned as a protocol, then reintroduced twice, in the 1900s and again in the 1990s (Viterbo et al. 1992). The underlying mechanism is controversial but is assumed to reflect collateral and/or terminal sprouting from the donor nerve in response to epineurotomy (and presumably perineurotomy). Current literature suggests that end-to-side neurorrhaphy should be regarded as a salvage procedure (Kettle et al. 2013) on the grounds that ...*“a discrepancy between experimental and clinical results still exists”*... (Tos et al. 2014).

In nerve transfer, the stump of all or part of a proximal nerve is coapted to the stump of a distal denervated nerve, typically to convert a high nerve injury to a lower level lesion closer to target muscles, with the aim of improving the possibility that those muscles will be reinnervated (Birch 2011; Tung 2014). Previously reserved for unsalvageable brachial plexus injuries (Tuttle 1913), an increased understanding of intraneural topography has seen nerve transfers used to repair a range of presentations, including proximal nerve root avulsion and multi-level nerve injuries (Moore and Novak 2014). Tuttle’s concluding remarks point to the future as he commends the technique for ...*“the results we may get in cases that seem almost hopeless, and the fact that even a long chance is worth taking”* (Tuttle 1913).

Entubulation (tubulisation) of nerve stumps using implantable nerve guidance conduits offers a non-neural option to repair transected nerves (Hall 2009). Early suture materials often provoked an inflammatory response at the suture site that impaired axonal regrowth into the distal nerve stump. The technique of entubulation that emerged towards the end of the nineteenth century promised a suture-less method of coapting nerve stumps. Themistocles Gluck is credited with the idea of using decalcified bone tubes (originally designed as wound drains by Gustav Neuber) as nerve conduits in 1880 (Ijpma et al. 2008): arteries, veins and rolls of fascia were also used to bridge long defects. The concept enjoyed a renaissance as an experimental tool in the 1960s. “Regeneration chambers” made of non-resorbable polymers were implanted between nerve stumps to explore the behavior of regrowing axons and their co-migrating Schwann cells as they crossed short interstump gaps (≤ 1 cm) (Williams et al. 1983). Experiments using inverted Y-shaped silicone-based conduits provided data that was factored into the design of experimental tissue-engineered devices containing Schwann cell-rich or stem cell-rich 3D scaffolds, intended to support and possibly enhance axonal regrowth across a long inter-stump gap. The use of empty synthetic nerve guidance tubes composed of collagen, polycaprolactone or polyglycolic acid subsequently translated to the clinic (e.g., Lundborg et al. 2004): more complex conduits, containing cell-doped internal scaffolds, remain preclinical.

Peripheral nerve injury is an under-appreciated clinical problem, even though it is more common than injury to the central nervous system. ... (Christie and Zochodne 2013)

Despite the long-standing evidence that peripheral neurons possess the capacity to regrow, unlike their counterparts in the CNS, ...*“less than half of patients who undergo nerve repair after injury regain good to excellent motor or sensory function and current surgical techniques are similar to those described by Sunderland more than 60 years ago”* (Grinsell and Keating 2014). Why are the results of nerve repair so often disappointing (Panagopoulos et al. 2017) when experimental research has for so long implied that it should be otherwise? Are current experimental methods fit for purpose? If that purpose is to explore the neurobiology of the injury response, then the answer is a qualified “yes.” However, their applicability to many clinical scenarios is less clear as the following four quotes eloquently make clear: ...*“Despite their great utility, there are inherent weaknesses of these rodent models that relate to their relatively short nerve graft lengths and exceptional neuroregenerative capacities...”* (Myckatyn and MacKinnon 2004); ...*“A “blow-through” effect is observed in rodents whereby an advancing nerve front overcomes an experimental defect given sufficient time, rendering experimental groups indistinguishable at late time points”...* (Brenner et al. 2008); ...*“a valid animal model of nerve regeneration requires it to reproduce the specific processes that take place in regeneration after human peripheral nerve injury. No distinct animal species meets all the requirements for an ideal animal model. Certain models are well suited for understanding regenerative neurobiology while others are better for pre-clinical evaluation of efficacy”* (Angius et al. 2012); ...*“young, healthy, research-bred rats that have strong regenerative potential and rapid healing rates, provide a very different regenerative paradigm to that of wounded, compromised and diverse human patients (in terms of wound healing, vascularity, susceptibility to infection, etc)”* (Kaplan et al. 2015).

The introduction of the operating microscope is hailed as one of the most significant advances in peripheral nerve reconstruction in the twentieth century, enabling ...*“a more accurate appraisal of nerve injuries and of their repair”...* (Smith 1964; Dvali and Mackinnon 2007). However, joining the stumps of a divided nerve together, or interposing a graft between the stumps, no matter how meticulous the microsurgery, cannot control the behavior of axons crossing a suture line or guarantee functional recovery (de Medinaceli and Seaber 1989). The size of many peripheral neurons (relative to all other cell types) confounds their successful recovery, not least because it adds the dimension of time to the list of factors that influence repair. Centrally, in addition to initiating immediate expression of regeneration-associated genes (RAGs) in the cell bodies of affected neurons, injury induces reprogramming of synaptic connectivity and triggers responses in affected dorsal root ganglia that may mediate the development and maintenance of chronic neuropathic pain. Peripherally, axonal regrowth over long distances is typically frustrated by progressive endoneurial fibrosis and Schwann cell senescence: the cardiologists’ aphorism “time is muscle” applies equally to the consequences of delaying nerve repair. The extent to which neighboring non-neural tissues such as bone and blood vessels are involved in the injury complicates exploration of the injury site and impairs regeneration (Woodhall 1947; Glasby et al. 1998; Fullarton et al. 1998; Birch et al. 2012). The age, health and compliance of the patient; and the

skill of the surgeon are additional confounding factors that influence outcome. Functional recovery is therefore often slow and incomplete: residual symptoms may include weakness, pain, dysaesthesia, impaired autonomic function, cold intolerance, and, when injuries affect the upper limb, lifelong impairment of hand function. There is an increased risk of secondary disability as a result of falls or fractures. Individuals may be unable to return to their previous employment or to any kind of profitable work; the economic burden to them, their carers, their dependents, and ultimately to the state is considerable (e.g., Ciaramitaro et al. 2010; Wojtkiewicz et al. 2015).

3 Conclusions

Despite four decades of research on nerve regeneration, clinical outcomes following peripheral nerve repair are unpredictable and often unsatisfactory. (Isaacs and Browne 2014)

Preventing neuronal death after axonal transection; encouraging regrowing axons and co-migrating Schwann cells to cross long inter-stump gaps; manipulating the cellular and molecular responses at the injury site and throughout the distal stump, to ensure the continuing “axon-responsiveness” of chronically denervated Schwann cells and end-organs; maintaining an adequate blood supply; minimizing axonal misdirection at the suture site and maximizing the accuracy of target reinnervation so that motor nerves grow to muscles and sensory nerves to skin and appropriate sensory organs; and abrogating neuropathic pain are some of the very real challenges that drive current bioscientific and clinical research. Therapeutic intervention locally cannot (yet) manipulate responses that may occur many centimeters from the injury and that may influence functional outcome months or even years afterwards.

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References

- Abdel-Halim RE (2001) Experimental medicine 1000 years ago. *Urol Ann* 3:55–61
- Angius D, Wang H, Spinner RJ, Gutierrez-Cotto Y, Yaszemski MJ, Windebank AJ (2012) A systematic review of animal models used to study nerve regeneration in tissue-engineered scaffolds. *Biomaterials* 33:8034–8039
- Arnemann J (1787) Versuche über die Regeneration an lebenden Tieren. I. Über die Regeneration der Nerven. Göttingen. Cited in Holmes 1951
- Arraez-Aybar LA, Bueno-Lopez JL, Raio N (2015) Toledo school of translators and their influence on anatomical terminology. *Ann Anat* 198:21–33
- Arthur-Farraj PJ, Latouche M, Wilton DK, Quintes S, Chabrol E, Banerjee A, Woodhoo A, Jenkins B, Rahman M, Turmaine M, Wicher GK, Mitter R, GreenSmith I, Behrens A, Raivich G, Mirsky R, Jessen KR (2012) C-Jun reprograms Schwann cells of injured nerves to generate a repair cell essential for regeneration. *Neuron* 75:633–647

- Artico M, Cervoni I, Nucci F, Giuffrè R (1996) Birthday of peripheral nervous system surgery: the contribution of Gabriele Ferrara (1543–1627). *Neurosurgery* 39:380–382
- Babcock WW (1927) A standard technique for operations on peripheral nerves. With especial reference to the closure of large gaps. *Surg Gynec Obstet* 45:364–378
- Bain JR, Mackinnon SE, Hudson AR, Wade J, Evans P, Makino A, Hunter D (1992) The peripheral nerve allograft in the primate immunosuppressed with Cyclosporin A: I. Histologic and electrophysiologic assessment. *Plast Reconstr Surg* 90:1036–1046
- Ballance CA Stewart P (1901) *The healing of nerves*. Macmillan and Co, London
- Barker CF, Markmann JF (2013) Historical overview of transplantation. *Cold Spring Harb Perspect Med* 3:a014977
- Bay NS, Bay BH (2010) Greek anatomist Herophilus: the father of anatomy. *Anat Cell Biol* 43:280–283
- Belen D, Aciduman A, Er U (2009) History of peripheral nerve repair: may the procedure have been practiced in Hippocratic School? *Surg Neurol* 72:190–194
- Birch R (2009) Causalgia: a restatement. *Neurosurgery* 65(4 Suppl):A222–A228
- Birch R (2011) *Surgical disorders of the peripheral nerves*, 2nd edn. Springer, London
- Birch R, Misra P, Stewart MP, Eardley WG, Ramasamy A, Brown K, Shenoy R, Anand P, Clasper J, Dunn R, Etherington J (2012) Nerve injuries sustained during warfare: part I—epidemiology. *J Bone Joint Surg Br* 94:523–528
- Borelli GA (1681) *De motu animalium*. Angelo Bernabo, Rome
- Boulleme AI (2016) The history of myelin. *Exp Neurol* 283:431–445
- Brenner MJ, Moradzadeh A, Myckatyn TM, Tung TH, Mendez AB, Hunter DA, Mackinnon SE (2008) Role of timing in assessment of nerve regeneration. *Microsurgery* 28:265–272
- Brooks DN, Weber RV, Chao JD, Rinker BD, Zoldos J, Robichaux MR, Ruggeri SB, Anderson KA, Bonatz EE, Wisotsky SM, Cho MS, Wilson C, Cooper EO, Ingari JV, Safa B, Parrett BM, Buncke GM (2012) Processed nerve allografts for peripheral nerve reconstruction: a multicenter study of utilization and outcomes in sensory, mixed, and motor nerve reconstructions. *Microsurgery* 32:1–14
- Bruch C (1855) Über die regeneration durchschnittenen nerven. *Zt wiss Zool* 6:135–138
- Brushart TM (1988) Preferential reinnervation of motor nerves by regenerating motor axons. *J Neurosci* 8:1026–1031
- Brushart TM (1993) Motor axons preferentially reinnervate motor pathways. *J Neurosci* 13:2730–2738
- Cajal SRY (1954) Neuron theory or reticular theory (trans: Purkiss MU, Fox CA). Consejo Superior De Investigaciones Científicas, Madrid
- Cajal SRY (1991) Degeneration and regeneration in the nervous system (trans: May R). Oxford University Press, Oxford
- Chalcidius (1876) *Platonis Timaeus Interprete Chalcidius cum Ejusdem Commentario*. B.G. Teubner, Lipsiae (Leipzig). [Cited in Ceesia GG (2012) Alcmaeon of Croton's observations on health, brain, mind, and soul. *J Hist Neurosci* 21:409–426]
- Chen P, Piao X, Bonaldo P (2015) Role of macrophages in Wallerian degeneration and axonal regeneration after peripheral nerve injury. *Acta Neuropathol* 130:605–618
- Christie KJ, Zochodne D (2013) Peripheral axon regrowth: new molecular approaches. *Neuroscience* 240:310–324
- Ciaramitaro P, Mondelli M, Logullo F, Grimaldi S, Battiston B, Sard A, Scarinzi C, Migliaretti G, Faccani G, Cocito D, Italian Network for Traumatic Neuropathies (2010) Traumatic peripheral nerve injuries: epidemiological findings, neuropathic pain and quality of life in 158 patients. *J Peripher Nerv Syst* 15:120–127
- Cruikshank WC (1795) Experiments on the nerves, particularly on their reproduction, and on the spinal marrow of living animals. *Phil Trans R Soc* 85, 512–519. (This paper dated 1795 records the experiment undertaken in 1776)
- Cruikshank W, Hunter J (1795) Experiments on the nerves, particularly on their reproduction and on the spinal marrow of living animals. *Philos Trans R Soc Lond* 85:177–189

- Dandy WE (1943) A method of restoring nerves requiring resection. *JAMA* 122:35–36
- de Medinaceli L, Seaber AV (1989) Experimental nerve reconnection: importance of initial repair. *Microsurgery* 10:56–70
- Decker L, Desmarquet-Trin-Dinh C, Taillebourg E, Ghislain J, Vallat JM, Charnay P (2006) Peripheral myelin maintenance is a dynamic process requiring constant Krox20 expression. *J Neurosci* 26:9771–9779
- Dellon AL (1992) Management of peripheral nerve injuries. Basic principles of microneurosurgical repair. *Oral Maxillofac Surg Clin N Am* 4:393–403
- Dobson JF (1927) Erasistratus. *Proc R Soc Med* 20:825–832
- Driscoll PJ, Glasby MA, Lawson GM (2002) An in vivo study of peripheral nerves in continuity: biomechanical and physiological responses to elongation. *J Orthop Res* 20:370–375
- Dubový P, Jančálek R, Kubek T (2013) Role of inflammation and cytokines in peripheral nerve regeneration. *Int Rev Neurobiol* 108:173–206
- Dvali L, Mackinnon S (2007) The role of microsurgery in nerve repair and nerve grafting. *Hand Clin* 23:73–81
- Ellis H (2009) The Cambridge illustrated history of surgery. Cambridge University Press, Cambridge
- Evans PJ, Midha R, Mackinnon SE (1994) The peripheral nerve allograft: a comprehensive review of regeneration and neuroimmunology. *Prog Neurobiol* 43:187–233
- Fish JS, Bain JR, McKee N, Mackinnon SE (1992) The peripheral nerve allograft in the primate immunosuppressed with Cyclosporin A: II. Functional evaluation of reinnervated muscle. *Plast Reconstr Surg* 90:1047–1052
- Ford BJ (1981) Enlightening neuroscience: microscopes and microscopy in the eighteenth century. In: Whitaker H, Smith CU, Finger S (eds) *Brain, mind and medicine: essays in eighteenth-century neuroscience*. Springer, Berlin, pp 29–41
- Ford BJ (1982) Antony van Leeuwenhoek's sections of *bovine* optic nerve. *Microscope* 30:171–184
- Foster M (1901) Lectures on the history of physiology during the 16th, 17th and 18th Centuries. Cambridge University Press, Cambridge
- Friedman AH (2009) An eclectic review of the history of peripheral nerve surgery. *Neurosurgery* 65(4 Suppl):A3–A8
- Fullerton AC, Glasby MA, Lawson GM (1998) Immediate and delayed nerve repair using freeze-thawed muscle allografts associated long-bone fracture. *J Hand Surg Br* 23:360–364
- Galvani L (1780) De viribus electricitatis in motu musculari commentaries. *Accademia dell Scienze, Bologna*
- Gebhart GF, Bielefeldt K (2016) Physiology of visceral pain. *Compr Physiol* 6:1609–1633
- Glasby MA, Fullerton AC, Lawson GM (1998) Immediate and delayed nerve repair using freeze-thawed muscle autografts in complex nerve injuries associated arterial injury. *J Hand Surg Br* 23:354–359
- Glenn TD, Talbot WS (2013) Signals regulating myelination in peripheral nerves and the Schwann cell response to injury. *Curr Opin Neurobiol* 23:1041–1048
- Glickstein M (2006) Golgi and Cajal: the neuron doctrine and the 100th anniversary of the 1906 Nobel Prize. *Curr Biol* 16:R147–R151
- Gomez-Sanchez JA, Carty L, Iruarizaga-Lejarreta M, Palomo-Irigoyen M, Varela-Rey M, Griffith M, Hantke J et al (2015) Schwann cell autophagy, myelinophagy, initiates myelin clearance from injured nerves. *J Cell Biol* 210(1):153–168
- Goodrich JT, Klot M (2015) History of the peripheral and cranial nerves. In: Tubbs RS et al (eds) *Nerves and nerve injuries*, vol 1. Elsevier, pp 3–22
- Gordon T (2014) Neurotrophic factor expression in denervated motor and sensory Schwann cells: relevance to specificity of peripheral nerve regeneration. *Exp Neurol* 254:99–108
- Graham JB, Neubauer D, Xue QS, Muir D (2007) Chondroitinase applied to peripheral nerve repair averts retrograde axonal regeneration. *Exp Neurol* 203:185–195
- Green CD (2003) Where did the ventricular localization of mental faculties come from? *J Hist Behav Sci* 39:131–142

- Grinsell D, Keating CP (2014) Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. *Biomed Res Int* 2014:698256
- Guillery RW (2007) Relating the neuron doctrine to the cell theory. Should contemporary knowledge change our view of the neuron doctrine? *Brain Res Rev* 55:411–421
- Günther AF, Schön JMA (1840) Versuche und Bemerkungen über Regeneration der Nerven und Abh Versuche und Bemerkungen über Regeneration der Nerven und Abhängigkeit der peripherischen Nerven von den Central-organen.ngigkeit der peripherischen Nerven von den Central-organen. *Müller's Archiv* 270–286
- Guthrie GJ (1827) A treatise on gunshot wounds, inflammation, erysipelas, and mortification, on injuries of the nerves, and on wounds of the extremities, requiring the different operation of amputation, 3rd edn. Burgess and Hill, London
- Haighton J (1795) An experimental inquiry concerning the reproduction of nerves. *Philos Trans R Soc Lond* 85:519–525
- Hall SM (1986a) Regeneration in cellular and acellular autografts in the peripheral nervous system. *Neuropathol Appl Neurobiol* 12:27–46
- Hall SM (1986b) The effect of inhibiting Schwann cell mitosis on the re-innervation of acellular autografts in the peripheral nervous system of the mouse. *Neuropathol Appl Neurobiol* 12:401–414
- Hall SM (1999) The biology of chronically denervated Schwann cells. *Ann N Y Acad Sci* 883:215–233
- Hall S (2009) Biomaterials for the repair of peripheral nerves. In: Di Sivio L (ed) *Cellular response to biomaterials*. Woodhead Publishing, Cambridge, pp 252–290
- Hanigan W (2010) The development of military medical care for peripheral nerve injuries during World War I. *Neurosurg Focus* 28(5):E24. <https://doi.org/10.3171/2010.3.FOCUS103>
- Harvey W (1628) *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus*. Frankfurt
- Henry FP, Goyal NA, David WS, Wes D, Bujold KE, Randolph MA, Winograd JM, Kochevar IE, Redmond RW (2009) Improving electrophysiologic and histologic outcomes by photochemically sealing amnion to the peripheral nerve repair site. *Surgery* 145:313–321
- Hernigou P (2013) Ambroise Paré II: Paré's contributions to amputation and ligature. *Int Orthop* 37:769–772
- Highe WB, Holmes W (1943) Traction injuries to the lateral popliteal nerve and traction injuries to peripheral nerves after suture. *Br J Surg* 30:212–233
- Highe WB, Sanders K (1943) The effects of stretching nerves after suture. *Br J Surg* 30:355–369
- Hirata K, Kawabuchi M (2002) Myelin phagocytosis by macrophages and nonmacrophages during Wallerian degeneration. *Microsc Res Tech* 57:541–547
- Hiscoe HB (1947) The distribution of nodes and incisures in normal and regenerated nerve fibres. *Anat Rec* 99:447–475
- Höke A, Sun HS, Gordon T, Zochodne DW (2001) Do denervated peripheral nerve trunks become ischemic? The impact of chronic denervation on vasa nervorum. *Exp Neurol* 172:398–406
- Höke A, Redett R, Hameed H, Jari R, Zhou C, Li ZB, Griffin JW, Brushart TM (2006) Schwann cells express motor and sensory phenotypes that regulate axon regeneration. *J Neurosci* 26:9646–9645
- Holmes W (1951) The repair of nerves by suture. *J Hist Med Allied Sci* 6:44–63
- Horn T (1946) The repair of peripheral nerve lesions. *Am J Surg* LXXII:489–493
- Howell WH, Huber GC (1892) A physiological, histological and clinical study of the degeneration and regeneration in peripheral nerve fibres after severance of their con nections with the nerve centres. Plates XII–XVII. *J Physiol* 13:335–406
- Howell WH, Huber GC (1893) A physiological, histological and clinical study of the degeneration and regeneration in peripheral nerve fibres after severance of their connections with the nerve centres. Part III. Critical résumé of surgical cases of primary and secondary suture. *J Physiol* 14:1–51
- Hunt T (1994) *Anglo-Norman medicine I Roger Frugard's Chirurgia and the Practica brevis of Platearius*. D S Brewer, Cambridge

- Ijpma FF, Van De Graaf RC, Meek MF (2008) The early history of tubulation in nerve repair. *J Hand Surg Eur* 33:581–586
- Ikeda M, Oka Y (2012) The relationship between nerve conduction velocity and fiber morphology during peripheral nerve regeneration. *Brain Behav* 2:382–390
- Ingebrigtsen R (1915) A contribution to the biology of peripheral nerves in transplantation. *J Exp Med* 22:418–426
- Isaacs J, Browne T (2014) Overcoming short gaps in peripheral nerve repair: conduits and human acellular nerve allograft. *Hand (N Y)* 9:131–137
- Jobe MT, Martinez SF (2012) Peripheral nerve injuries. In: Canale ST, Beaty JH (eds) *Campbells' operative orthopaedics* e-book, part XVI, chapter 62, vol IV. Elsevier, Amsterdam, pp 3062–3126
- Jobe MT, Martinez SF (2013) Peripheral nerve injuries. In: Canale, Beaty (eds) *Campbell's operative orthopaedics*, 12 edn. Elsevier, Philadelphia, pp 3063–3065. Ch 62D
- Jones EG (1999) Golgi, Cajal and the neuron doctrine. *J Hist Neurosci* 8:170–178
- Ju MS, Lin CC, Fan JL, Chen RJ (2006) Transverse elasticity and blood perfusion of sciatic nerves under in situ circular compression. *J Biomech* 39:97–102
- Kaplan HM, Prakhar M, Kohn J (2015) The overwhelming use of rat models in nerve regeneration research may compromise designs of nerve guidance conduits for humans. *J Mater Sci Mater Med* 26:226
- Kawamura DH, Johnson PJ, Moore AM, Magill CK, Hunter DA, Ray WZ, Tung TH, Koeppen AH (2004) Wallerian degeneration: history and clinical significance. *J Neurol Sci* 220:115–117
- Kettle SJ, Starritt NE, Glasby MA, Hems TE (2013) End-to-side nerve repair in a large animal model: how does it compare with conventional methods of nerve repair. *J Hand Surg Eur* 38:192–202
- Koeppen AH (2004) Wallerian degeneration: history and clinical significance. *J Neurol Sci* 220:115–117
- Kokkalis ZT, Pu C, Small GA, Weiser RW, Venouziou AI, Sotereanos DG (2011) Assessment of processed porcine extracellular matrix as a protective barrier in a rabbit nerve wrap model. *J Reconstr Microsurg* 27:19–28
- Létiévant E (1873) *Traité des Sections Nerveuses*. Baillière, Paris
- Leuzzi S, Armenio A, Leone L, De Santis V, Di Turi A, Annoscia P, Bufano L, Pascone M (2014) Repair of peripheral nerve with vein wrapping. *G Chir* 35:101–106
- Li H, Terenghi G, Hall SM (1997) Effects of delayed re-innervation on the expression of c-erbB receptors by chronically denervated rat Schwann cells in vivo. *Glia* 20:333–347
- Little KM, Zomorodi AR, Selznick LA, Friedman AH (2004) An eclectic history of peripheral nerve surgery. *Neurosurg Clin N Am* 15:109–123
- Lloyd BM, Luginbuhl RD, Brenner MJ, Rocque BG, Tung TH, Myckatyn TM, Hunter DA, Mackinnon SE, Borschel GH (2007) Use of motor nerve material in peripheral nerve repair with conduits. *Microsurgery* 27:138–145
- Lundborg G (1988) Intraneural microcirculation. *Orthop Clin North Am* 19:1–12
- Lundborg G, Rosen B, Dahlin L, Holmberg J, Rosen I (2004) Tubular repair of the median or ulnar nerve in the human forearm: a 5-year follow-up. *J Hand Surg Br* 29:100–107
- Mackinnon SE (2010) Matching of motor-sensory modality in the rodent femoral nerve model shows no enhanced effect on peripheral nerve regeneration. *Exp Neurol* 223:496–504
- Mackinnon SE, Dellon AL (1988) Nerve repair and nerve grafts. In: Mackinnon SE (ed) *Surgery of the peripheral nerve*. Thieme, New York
- Mackinnon SE, Dellon AL, O'Brien JP (1991) Changes in nerve fiber numbers distal to a nerve repair in the rat sciatic nerve model. *Muscle Nerve* 14:1116–1122
- Madison RD, Robinson GA, Chadaram SR (2007) The specificity of motor neurone regeneration (preferential reinnervation). *Acta Physiol (Oxf)* 189:201–206
- Mayo-Robson AW (1917) Nerve grafting as a means of restoring function in limbs paralysed by gunshot or other injuries. *Br Med J* 1(2926):117–118
- Mazzarello P (1999) A unifying concept: the history of cell theory. *Nat Cell Biol* 1:E13–E15

- Millesi H (1986) The nerve gap. Theory and clinical practice. *Hand Clin* 2:651–663
- Moore AM, MacEwan M, Santosa KB, et al. (2011) Acellular nerve allografts in peripheral nerve regeneration: a comparative study. *Muscle Nerve*. 44:221–234
- Moore AM, Novak CB (2014) Advances in nerve transfer surgery. *J Hand Ther* 27:96–104
- Moosavi J (2009) The place of Avicenna in the history of medicine. *Avicenna J Med Biotechnol* 1:3–8
- Myckatyn TM, Mackinnon SE (2004) A review of research endeavors to optimize peripheral nerve reconstruction. *Neurol Res*. 26:124–138
- Myers RR, Murakami H, Powell HC (1986) Reduced nerve blood flow in edematous neuropathies: a biomechanical mechanism. *Microvasc Res* 32:145–151
- Nasse CF (1839) Über die Veränderungen der Nervenfasern nach ihrer Durchschneidung. *Müller's Archiv* 405–419
- Neubauer D, Graham JB, Muir D (2010) Nerve grafts with various sensory and motor fiber compositions are equally effective for the repair of a mixed nerve defect. *Exp Neurol* 223:203–206
- Nichols CM, Brenner MJ, Fox IK, Tung TH, Hunter DA, Rickman SR, Mackinnon SE (2004) Effects of motor versus sensory nerve grafts on peripheral nerve regeneration. *Exp Neurol* 190:347–355
- Ochs S (1977) The early history of nerve regeneration beginning with Cruikshank's observations in 1776. *Med Hist*. 21:261–274
- Orf G (1981) What governs the size of the retraction gap in divided peripheral nerves. *Neurosurg Rev* 4:11–16
- Panagopoulos GN, Megaloikonon PD, Mavrogenis AF (2017) The present and future for peripheral nerve regeneration. *Orthopedics* 40:e141–e156
- Paré A (1634) The works of that famous chirurgion Ambrose Parey translated out of Latine and compared with the French. T. Johnson. Tr, London [Cited in Holmes 1951]
- Parkinson DB, Bhaskaran A, Droggiti A, Dickinson S, D'Antonio M, Mirsky R, Jessen KR (2004) Krox-20 inhibits Jun-NH2-terminal kinase/c-Jun to control Schwann cell proliferation and death. *J Cell Biol* 164:385–394
- Parkinson DB, Bhaskaran A, Arthur-Farraj P, Noon LA, Woodhoo A, Lloyd AC, Feltri ML, Wrabetz L, Behrens A, Mirsky R, Jessen KR (2008) c-Jun is a negative regulator of myelination. *J Cell Biol* 181:625–637
- Pearce JMS (2008) The Development of Spinal Cord Anatomy *Eur Neurol* 59:286–291
- Pellitteri R, Cova L, Zaccheo D, Silani V, Bossolasco P (2016) Phenotypic modulation and neuroprotective effects of olfactory ensheathing cells: a promising tool for cell therapy. *Stem Cell Rev* 12:224–234
- Perroncito A (1905) Sulla questione della rigenerazione autogena della fibre nervosa. *Boll. Soc. Med. Chir. Pavia* 360–363
- Philipeaux J, Vulpian A (1859) Notre sur des expériences démontrant que les nerfs séparés des centres nerveux peuvent après être altérés complètement se régénérer tout en demeurant isolés de des centres et recouvrir leurs propriétés physiologiques. *C R Hebd Acad Sci (Paris)* 59:507–509
- Philipeaux, J.-M., Vulpian A (1860) Recherches expérimentales sur la régénération des nerfs séparés des centres nerveux. *Comptes rendus des séances et mémoires de la Société de Biologie. C R séances et mémoires de la Société de Biologie*, 1:1–77.
- Prévost P (1826) Note sur la régénération du tissu nerveux. *Mém Soc Phys Genève*, 3 (cited in Holmes W (1951) The repair of nerves by suture. *J Hist Med Allied Sci* 6:44–63)
- Provencher M T, Abdu W A (2000) Giovanni Alfonso Borelli: "Father of spinal biomechanics." *Spine*. 25:131–136
- Ranvier LA (1878) Leçons sur l'Histologie su Système Nerveux. Librairie F. Savy, Paris
- Rao M, Nelms BD, Dong L, Salinas-Rios V, Rutlin M, Gershon MD, Corfas G (2015) Enteric glia express proteolipid protein 1 and are a transcriptionally unique population of glia in the mammalian nervous system. *Glia*. <https://doi.org/10.1002/glia.22876>. [Epub ahead of print]

- Remak R (1838) *Observationes anatomicæ et microscopicæ de systematis nervosi structura*. Berolini, Reims
- Rinker B, Zoldos J, Weber RV, Ko J, Thayer W, Greenberg J, Leversedge FJ, Safa B, Buncke G (2017) Use of processed nerve allografts to repair nerve injuries greater than 25 mm in the hand. *Ann Plast Surg* 78(6S Suppl 5):S292–S295
- Sadek AF, Fouly EH, Hamdy M (2014) Functional and electrophysiological outcome after autogenous vein wrapping of primary repaired ulnar nerves. *Microsurgery* 34:361–366
- Saheb-Al-Zamani M, Yan Y, Farber SJ, Hunter DA, Newton P, Wood MD, Stewart SA, Johnson PJ, Mackinnon SE (2013) Limited regeneration in long acellular nerve allografts is associated with increased Schwann cell senescence. *Exp Neurol* 247:165–177
- Salzer JL (2008) Switching myelination on and off. *J Cell Biol* 181:575–577
- Schmidt G (1993) Eduard Albert and the beginning of human nerve grafting. *Acta Chir Austriaca* 25:287–288
- Schuetze SM (1983) The discovery of the action potential. *Trends Neurosci* 6:164–168
- Seddon HJ (1942) A classification of nerve injuries. *Br Med J* 2:237–239
- Shoja MM, Tubbs RS (2007) The history of anatomy in Persia. *J Anat* 210:359–378
- Simpson D (2009) From Lanfranc to Sunderland: the surgery of peripheral nerve injuries. *ANZ J Surg* 79:930–935
- Smith JW (1964) Microsurgery of peripheral nerves. *Plast Reconstr Surg* 33:317–329
- Souayah N, Greenstein JI (2005) Insights into neurologic localization by Rhazes, a medieval Islamic physician. *Neurology* 65:125–128
- Stranding S (2016) A brief history of topographical anatomy. *J Anat* 229:32–62
- Stassart RM, Fledrich R, Velanac V, Brinkmann BG, Schwab MH, Meijer D, Sereda MW, Nave KA (2013) A role for Schwann cell-derived neuregulin-1 in remyelination. *Nat Neurosci* 16:48–54
- Steinrück CO (1838) *De nervorum regeneratione*. Decker, Berlin
- Sulaiman OA, Gordon T (2000) Effects of short- and long-term Schwann cell denervation on peripheral nerve regeneration, myelination and size. *Glia* 32:234–246
- Sunderland S (1945a) The intraneural topography of the radial, median, and ulnar nerves. *Brain* 68:243–299
- Sunderland S (1945b) The adipose tissue of peripheral nerves. *Brain* 68:118–122
- Sunderland S (1951) A classification of peripheral nerve injuries producing loss of function. *Brain* 4:491–516
- Swan J (1820) *A dissertation on the treatment of morbid local affections of nerves*. Drury, London
- Sykes AH (2000) Wallerian degeneration. In: Koehler PJ, Bruyn GW, Pearce JM (eds) *Neurological eponyms*. Oxford University Press, Oxford, pp 63–70
- Szynkaruk M, Kemp SW, Wood MD, Gordon T, Borschel GH (2013) Experimental and clinical evidence for use of decellularized nerve allografts in peripheral nerve gap reconstruction. *Tissue Eng Part B Rev* 19:83–96
- Terzis JK, Sun DD, Thanos PK (1997) Historical and basic science review: past, present, and future of nerve repair. *J Reconstr Microsurg* 13:215–225
- Tinel J (1917) *Nerve wounds. Symptomatology of peripheral nerve lesions caused by war wounds* (eds and trans: Rothwell F, Joll CA). William Wood and Company, New York
- Topp KS (2015) Nerve biomechanics. In: Stranding S (ed) *Gray's anatomy. The anatomical basis of clinical practice*, 41st edn. Elsevier, Amsterdam. Commentary 9.1
- Tos P, Colzani G, Ciclamini D, Titolo P, Pugliese P, Artiaco S (2014) Clinical applications of end-to-side neurorrhaphy: an update. *Biomed Res Int* 2014:646128
- Trumble TE, McCallister WV (2000) Repair of peripheral nerve defects in the upper extremity. *Hand Clin* 16:37–52
- Tsao JW, George EB, Griffin JW (1999) Temperature modulation reveals three distinct stages of Wallerian degeneration. *J Neurosci* 19:4718–4726
- Tung TH (2014) Nerve transfers. *Clin Plast Surg* 41:551–559
- Tuttle H (1913) Exposure of the brachial plexus with nerve transplantation. *JAMA* 61:15–17

- Ushiki T, Ide C (1990) Three-dimensional organization of the collagen fibrils in the rat sciatic nerve as revealed by transmission- and scanning electron microscopy. *Cell Tissue Res* 260:175–184
- Vargas ME, Watanabe J, Singh SJ, Robinson WH, Barres BA (2010) Endogenous antibodies promote rapid myelin clearance and effective axon regeneration after nerve injury. *Proc Natl Acad Sci U S A* 107:11993–11998
- Viterbo VF, Trindade JC, Hoshino K, Mazzoni Neto A (1992) Latero-terminal neuroraphy without removal of the epineural sheath. Experimental study in rats. *Rev Paul Med* 110:267–275
- Vizoso AD, Young JZ (1948) Internode length and fibre diameter in developing and regenerating nerves. *J Anat* 82:110–134
- von Fleischhacker R (1894) *Lanfrank's science of Chirurgie by Lanfranco, of Milan*. Published for the Early English Text Society by Kegan Paul, Trench, Trübner, London
- Vulpian EFA (1866) *Leçons sur la Physiologie Générale et Comparée du Système Nerveux*. Cited in: De Felipe J, Jones EG (1991) Introduction to: Cajal's degeneration and regeneration of the nervous system. Oxford University Press, Oxford
- Waldeyer-Hartz HWG (1891) Über einige neuere Forschungen im Gebiete der Anatomie des Zentralnervensystems. *Deutsche Med. Wochenschr* 17:1213–1218, 1244–1246, 1267–1269, 1287–1289, 1331–1332, 1352–1356. [Cited in Guillery RW (2007) Relating the neuron doctrine to the cell theory. Should contemporary knowledge change our view of the neuron doctrine? *Brain Res Rev* 55:411–421]
- Waller AV (1850) Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog and observations of the alterations produced thereby in the structure of their primitive fibres. *Philos Trans R Soc Lond* 140:423–429
- Waller AV (1852) Nouvelles recherches sur la regeneration des fibres nerveuses. *C R Hebd Acad Sci (Paris)* 34:675–679
- Walters BC (2015) History of peripheral nerve repair. In: Tubbs RS, Rizk E, Shoja MM, Loukas M, Barbaro N, Spinner RJ (eds) *Nerves and nerve injuries*. Elsevier, Amsterdam, pp 23–36
- Wang JT, Medress ZA, Barres BA (2012) Axon degeneration: molecular mechanisms of a self-destruction pathway. *J Cell Biol* 196:7–18
- Wickens A (2015) *A history of the brain*. Psychology Press, London
- Williams LR, Longo FM, Powell HC, Lundborg G, Varon S (1983) Spatial-temporal progress of peripheral nerve regeneration within a silicone chamber: parameters for a bioassay. *J Comp Neurol* 218:460–470
- Williams HB, Jabaley ME (1986) The importance of internal anatomy of the peripheral nerves to nerve repair in the forearm and hand. *Hand Clin* 2:689–707
- Wiltse LL, Pait GT (1998) Herophilus of Alexandria (325–255B.C.): the father of anatomy. *Spine* 23:1904–1914
- Wojtkiewicz DM, Saunders J, Domeshek L, Novak CB, Kaskutas V, Mackinnon SE (2015) Social impact of peripheral nerve injuries. *Hand (N Y)* 10:161–167
- Woodhall B (1947) Peripheral nerve injuries; basic data from the peripheral nerve registry concerning 7,050 nerve sutures and 67 nerve grafts. *J Neurosurg* 4:146–163
- Yang H, He BR, Hao DJ (2015) Biological roles of olfactory ensheathing cells in facilitating neural regeneration: a systematic review. *Mol Neurobiol* 51:168–179
- Yi C, Dahlin LB (2010) Impaired nerve regeneration and Schwann cell activation after repair with tension. *Neuroreport* 21:958–962

Part I

The Peripheral Nerve Repair Environment



Blood Supply and Microcirculation of the Peripheral Nerve

Cosima Prahm, Johannes Heinzel, and Jonas Kolbensschlag

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Abstract

The aim of this chapter is to comprehensively compile the current body of knowledge relating to the blood supply and microcirculation of the peripheral nerve. Key findings are summarized to convey to readers which important aspects have been discovered and which further studies could be of special interest. The complex anatomy of the peripheral nerve's microcirculatory system, its physiology, and pathophysiology as well as its crucial involvement in nerve regeneration are discussed. Special emphasis is placed on the lymphatic system, the involvement of which in peripheral nerve injury and regeneration remains to be elucidated. This chapter focuses on experimental concepts and emerging techniques to deepen our understanding of the peripheral nerve vascular system, both in regard to its function and how it could be modified to enhance nerve regeneration. It concludes with an outlook on clinical applications to improve peripheral nerve (re)vascularization, ranging from vascularized nerve grafts and surgical angiogenesis to bioengineered conduits and the use of stems cells. With the help of this chapter, researchers interested in tissue-engineering will be provided with a broad fundament of knowledge, intended as an aid for the development of new approaches to improve peripheral nerve regeneration.

1 Introduction

The microcirculatory system of the peripheral nerve, much as in other tissues such as muscle, is responsible for providing oxygenated blood and elimination of waste products via the blood stream. For obvious reasons, the conductive system of the peripheral nerve has been the primary interest of researchers from various disciplines. However, for this to function properly, an intact blood supply is an important prerequisite.

Therefore, the aim of this chapter is to give the reader an overview of the anatomical and physiological basis of peripheral nerve microcirculation and how they were discovered. From there, potential clinical issues that go along with an impaired perfusion of the nerve and current treatment options are discussed. An

outlook on future perspectives in this field aims to discuss problems that are not yet or not sufficiently addressed and stipulate future research in this promising area.

2 Historical Overview

The first written treatise concerned exclusively with the vascular system of peripheral nerves is widely considered to be “De Vasis Nervorum,” which was published in 1768 (Doerffler 1768). It is the medical doctoral thesis of Johannes Doerffler, supervised by Jacob Isenflamm at the University of Erlangen, Germany. Doerffler injected fluid wax into the major vessels from which he traced and described the branches running along and toward the main nerves. Since his pioneering work, several morphological and physiological studies from the seventeenth to the twenty-first century have brought further insight into the anatomy and physiology of the vasa nervorum and their impact on nerve function.

Josef Hyrtl, a famous anatomist from Vienna, published one of his most prominent books on the human anatomy in 1846 (Hyrtl 1846). While being a mainstay of human anatomy in general, it was also rich in information of the vascular system of the peripheral nerves in particular. In 1894, Quénu and Lejars published their work called “Études sur le système circulatoire” (Quénu and Lejars 1894). While it dealt not exclusively with the vessels of the peripheral nerves, it depicted them in great detail. It is one of the first works to describe the intrafascicular patterns of peripheral nerve blood supply as well as the relationship between cutaneous nerves and vessels.

An excellent discussion on this topic can also be found in the work of W. Adams (1942, 1943). Sir Sydney Sunderland, one of the founding fathers of peripheral nerve research, also published extensively on the vasa nervorum (Sunderland 1945a, b, c).

German anatomist Johannes Lang from Würzburg conducted studies regarding the connective tissue and vessels of the peripheral nerves. On the one hand, he described the *conjunctiva nervorum* (Lang 1962), which was later referred to as the “paraneurium” by Millesi and thought to be paramount for the gliding of peripheral nerves (Millesi et al. 1990, 2007). On the other hand, Lang also published a comprehensive analysis of the course of the vasa nervorum and their relationship to the other components of a peripheral nerve, with special emphasis on their growth during fetal development (Lang 1964).

Göran Lundborg can be credited with being one of the major researchers and surgeons to take this accumulated knowledge and transfer it into clinical applications. For example, he showed that one extrinsic vessel of a nerve was able to provide sufficient blood to a length to diameter ratio of 45:1 (Lundborg 1988). Based on his work, Maki et al. (1993, 1997) later found that this can even be extended to 63:1 based on the proximal blood supply. Lundborg, among others, also described the circulatory response of these vessels to trauma and compression in great detail. He also showed that an elongation of the nerve due to tension on the coaptation site of only 8% significantly reduced venous flow. Only 15% of elongation led to a complete standstill in microcirculation. However, after release of the tension, the nerve was able to recover and regain adequate blood flow. Hanno Millesi took these

findings to the clinical setting, as he was one of the foremost proponents of a tension-free nerve coaptation. To accomplish this, he advocated the use of autologous nerve grafts and was able to achieve impressive results at a time where the results of nerve repairs were often mediocre (Millesi 2000, 2007).

Given this pivotal role of blood supply for peripheral nerve function and the knowledge of their extrinsic vascular anatomy, it is only consequential that researchers developed the idea of vascularized autologous nerve grafts. Use of a vascularized nerve graft was pioneered by Strange, who used a pedicled segment of the ulnar nerve to bridge a defect of the median nerve in 1945 (Strange 1947). Ian Taylor did not only revolutionize reconstructive microsurgery by his description of free tissue transfers based on the angiosome concept (Taylor and Daniel 1973), but was also the first to report the transfer of a free vascularized nerve graft in 1976 (Taylor and Ham 1976). Taylor was also the first to report five distinct types of neurovascular bundles considering both the anatomical features of the nerve as well as of its supplying vessels (Taylor 1978). This classification system was later expanded and refined by Breidenbach and Terzis (1986) as well as by El-Barrany et al. (1999).

These researchers, among many others, gave us an understanding of the anatomy and physiology of the peripheral nerve and its blood supply. Standing on the shoulders of these giants enables us to push the boundaries of peripheral nerve reconstruction and regeneration even further.

3 Embryology and Anatomy

Peripheral nerves, as any other organ, rely on blood supply to meet their physical and functional needs. Throughout the human body, the regular distance between a cell and the nearest capillary supplying it is within the range of 1/10–1/5 mm (Jain et al. 2005; Muangsant et al. 2018). There are no major arteries that specifically cater to supply nutrients to nerves, instead, each nerve receives a branch stemming from adjacent arteries (Durward 1948). The plexus of small arteries providing blood supply to the peripheral nerves from adjacent blood vessels into the nerve itself are called the vasa nervorum.

Although they extend longitudinally along the nerve, they form numerous anastomoses between and within the connective tissue of the peripheral nerve, the epi-, peri-, and endoneurium, reinforcing the blood supply and maintaining a homeostatic environment (Lagerlund and Low 1993; Caillaud et al. 2019; Reinhold and Rittner 2020; Frueh et al. 2020).

3.1 Embryology of the Peripheral Neurovascular System

The close relationship between blood vessels and peripheral nerves is mirrored by their closely connected embryonic development despite the different germ layer sources of their progenitor cells (Brunet et al. 2014). Angioblasts, the precursors of the vascular system's endothelial cells, stem from the extraembryonic mesoderm and

embryonic splanchnic mesoderm whereas most neurons and glial cells of the peripheral nervous system are derived from the neural plate, a layer of the ectoderm (Segarra et al. 2019). It was shown that axonal projections of neurons follow vascular tracts, e.g., endothelin signaling is involved in guiding neurons of the superior cervical ganglia along the carotid arteries (Makita et al. 2008; Segarra et al. 2019). During embryonic development, newly formed vascular and neural structures penetrate the mesenchyme in parallel to provide both sufficient innervation and blood supply to the newly formed organs. This close relationship has coined the term “neurovascular bundle” while the parallel alignment of both systems is referred to as “neurovascular congruence” (Martin and Lewis 1989; Bates et al. 2003; Stubbs et al. 2009; Segarra et al. 2019). Studies utilizing embryonic chicken provided the finding that axonal growth is not predominantly guided by vasculature during early stages of development although the latter precedes the former. For later developmental stages two mechanisms of neurovascular inter-coordination have been suggested. Whereas on the one hand neuronal and vascular alignment can be guided by intrinsic signals released from the mesenchyme, the direction of vascular growth is influenced by peripheral nerve routing on the other hand (Martin and Lewis 1989; Segarra et al. 2019). Using a mouse model, Mukouyama et al. found that arterial but not venous vessels are guided by the release of vascular endothelial growth factor (VEGF) released from either sensory nerve fibers or the Schwann cells surrounding them (Mukouyama et al. 2002, 2005). Li and colleagues found that the chemokine CXCL12, which is released from neuronal cells, is involved in the establishment of the neurovascular bundle. Several other potential molecules and pathways have been identified as key players for neurovascular congruence but discussing them all in detail would go far beyond the scope of this chapter (Madison et al. 2000; Bates et al. 2003; Glebova and Ginty 2005; Castets and Mehlen 2010; James and Mukouyama 2011; Li et al. 2013; Chauvet and Mann 2013; Oh and Gu 2013; Eichmann and Brunet 2014; Segarra et al. 2019).

Particularly noteworthy is the finding that semaphorin-3A, neuropilin-1, and plexin-A1, which are involved in dendritic cell maturation and axonal guidance, are also essential for lymphatic vessel maturation and valve formation (Takamatsu et al. 2010; Bouvrée et al. 2012; Jurisic et al. 2012). Additionally, it was shown that nerve growth factor (NGF), is a strong mediator of lymphangiogenesis and also stimulates release of VEGF-C, which in turn promotes lymphangiogenesis (Aloe et al. 2012; Sweat et al. 2014; Fink et al. 2014). These findings indicate that not only the development of the arterial and venous vascular system but also the lymphatic system is closely linked with neural development and maturation (Frueh et al. 2020).

The neurovascular congruence plays a pivotal role during nerve regeneration, in which Schwann cell migration is preceded by endothelial cell migration and subsequent vascularization. The peripheral nervous system's glial cells then use the newly formed vessels as scaffold before they in turn guide the regenerating nerve's axonal sprouts to their respective target organ (Parrinello et al. 2010; Cattin et al. 2015). In the light of this “vasophilic migration of neural cells” (Segarra et al. 2015), it seems only logical that both the vascular and neuronal cell systems start to interact already during early phases of development (Takahashi et al. 2013; Segarra et al. 2019).

3.2 Extrinsic and Intrinsic System

The vasa nervorum comprises of two functionally independent systems: The extraneural and intraneural systems. The extraneural system is located outside the epineurium, where the peripheral nerve is supplied by regional extrinsic vessels (Best and Mackinnon 1994). The extrinsic system consists of rather short vessels, varying in length between 50 and 250 mm (Sunderland 1978). These vessels are necessarily meandering in order to allow the peripheral nerves sufficient mobility during translational movement. An increase of branching vessels can be found especially in the vicinity of joints (Stanton-Hicks 2018). Taylor described five types of neurovascular bundles and also rated these supply patterns regarding the suitability for a vascularized nerve transfer. However, for his classification system he both considered the vascular anatomy as well as the nerve's branching pattern, with the purpose to define the optimum neurovascular bundle for vascularized nerve transfer (Taylor 1978).

Breidenbach and Terzis described their findings of blood supply of peripheral nerves after extensive microdissection, initially reporting five patterns (Breidenbach and Terzis 1984) which were later narrowed down to three for the sake of simplicity (Breidenbach and Terzis 1986). Summarizing their discoveries, they found that nerve could either be supplied by: (I) numerous small arterial branches but not by a dominant pedicle (II) one dominant arterial pedicle, or (III) multiple dominant arteries (Breidenbach and Terzis 1987).

El-Barrany et al. later refined the aforementioned authors' five-point scale after dissection of 30 cadaveric specimens.

Five patterns (Fig. 1) were identified. (I) No dominant arterial pedicle, (II) One dominant arterial pedicle; (III) One dominant arterial pedicle branching into

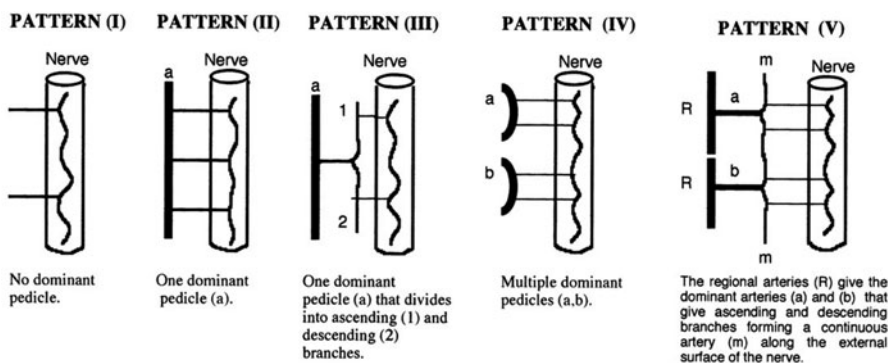


Fig. 1 Diagram of possible patterns of blood supply to the peripheral nerves. (I) No dominant pedicle. (II) One dominant pedicle. (III) One dominant pedicle (a) that divides into ascending (1) and descending (2) branches; (IV) Multiple dominant pedicles; (V) The regional arteries (R) give the dominant arteries (a) and (b) that give ascending and descending branches forming a continuous artery (m) along the external surface of the nerve. (Adopted with permission from El-Barrany et al. (1999))

ascending and descending branches; (IV) Multiple dominant arterial pedicles; (V) Regional arterial pedicles that form dominant arteries, which in turn branch off in ascending and descending branches forming a continuous artery (El-Barrany et al. 1999).

In an irregular sequence, radicular vessels branch off from these extraneural arteries and supply the intrinsic microvascular network of the vasa nervorum. Radicular vessels thereby connect the extrinsic components with the intrinsic longitudinal vasculature found within the nerve.

After this primary branching, a repeated branching of vessels can be observed, with the finest vessels penetrating deeper into the endoneurium within the fasciculi and finally forming a longitudinally oriented capillary bed with numerous anastomoses, the transperineurial arteriole (Durward 1948; Sunderland 1978; Lundborg 1988; Boissaud-Cooke et al. 2015). The transperineurial vessels penetrate the perineurium at a right angle, making them especially susceptible to kinking when the perineurium is stretched (Myers et al. 1986; Firrell 2019) (Figs. 2 and 3).

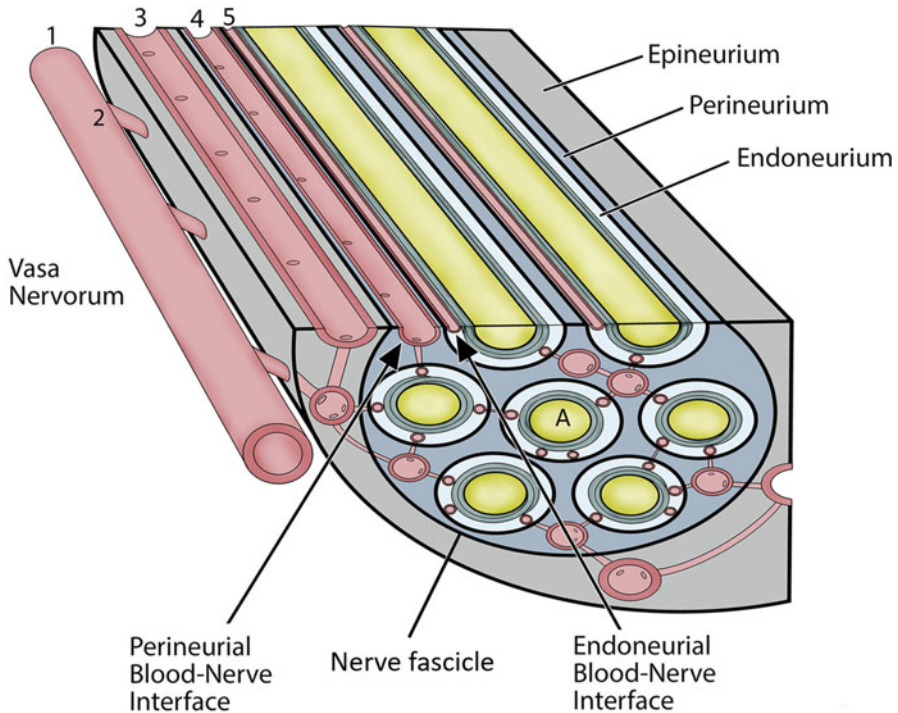


Fig. 2 The vasa nervorum and the blood–nerve interface. The extrinsic vessels (1), originating from main arteries, branch into radicular vessels (2) that anastomose with the intrinsic microvascular network supplying nutrients to the nerve. The epineurial vasculature (3) is longitudinally aligned, following the nerve fascicles, branching off again into the subcutaneous perineurial (4) and endoneurial capillaries (5) encompassing the axon (A). There are multiple anastomoses between these vessels. (Adapted with permission from Gugala et al. (2018))

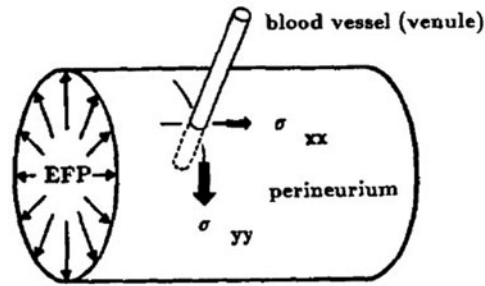


Fig. 3 Schematic drawing of a transperineurial vessel (venule) and its course through the perineurium. A 50- μ m-diameter venule is shown traversing the perineurial sheath. The drawing is not to scale; the ratio of the vessel diameter to perineurial diameter is 1:40. The vessel enters the perineurial shell at a right angle and is deformed by stretching of the perineurium which is distended by increased tissue fluid pressure (endoneurial fluid pressure, EFP) secondary to the accumulation of edema, e.g., in case of peripheral neuropathy. Plane strain analysis incorporating EFP with the biomechanical properties of the perineurium produces a ratio for σ_{yy} to σ_{xx} ranging from 5 to 10. EFP is an important factor in structural analyses since its variation within the pathophysiologic range of 2–8 mm Hg will produce unequal stresses in the circumferential (y) and longitudinal (x) axes. (Adopted with permission from Myers et al. (1986))

3.3 The Blood–Nerve Interface

The connective tissues forming the outer layers of epi-, the inner peri- and the innermost endoneurium not only function as a mechanical protection, but also as a restrictive, impermeable barrier actively regulating the vascular supply. It can be described as a dynamic interface consisting of the capillary endothelium, cells of the endoneurium and the multilayered cellular envelope of the perineurium, forming a separately regulated homeostatic compartment made accessible through tight junctions (Boissaud-Cooke et al. 2015; Gugala et al. 2018; Ubogu 2020). The endoneurial tissue is enveloping axons and thus directly interfaces myelin and Schwann cells of the peripheral nerves (Gugala et al. 2018). The perineurial and endoneurial vessels form an interconnected microcirculation network which maintains a regulated hydrostatic pressure of the endoneurial fluid. The endothelial cells of the perineurial and endoneurial capillaries are tightly connected by junction molecules, creating narrow and highly regulatory interfaces where the nerve interacts with the systemic circulation. Therefore, these two perineurial and endoneurial interfaces form a structural and functional continuum - the blood–nerve interface or blood–nerve barrier (Gugala et al. 2018). In turn, the arterioles of the epineurium feature open endothelial clefts and are therefore permeable to a variety of substances and tracers (Gugala et al. 2018). The human blood–nerve barrier, similar to other microvascular systems such as the blood–brain barrier, is expected to have high transendothelial electrical resistance, low permeability to solutes and macromolecules, and low transendothelial water flow (hydraulic conductivity). Comparative mammalian animal studies have shown that, after the blood–brain barrier, the blood–nerve barrier is the second most restrictive microvascular tissue barrier in mammals (Rechthand et al. 1987; Poduslo et al. 1988, 1994, 1998; Poduslo and Curran 1992; Helton et al. 2017; Ubogu 2020).

3.4 The Lymphatic System of the Peripheral Nerve

In contrast to the arterial and venous vessels running along and within all of the peripheral nerve segments and enveloping sheaths, lymphatic vessels have exclusively been located in the external epineurium (Shdanow 1931; Sunderland 1965). Knowledge regarding the anatomy and physiology of lymphatics within the peripheral nervous system is still very sparse, although it was hypothesized that lymphatic vessels may play a pivotal role in the pathophysiology of neural fibrosis, one of the hallmarks of peripheral nerve injury (Millesi 1992; Frueh et al. 2020). Early work published in the early 20th century pointed to the curious finding that bacteria and dyes can migrate along and within peripheral nerves and perineural lymphatics, but cannot penetrate or enter the liquor or spinal cord (Thiele and Embleton 1914). In the following decades, only a few papers added to the understanding of the lymphatic system of the peripheral nerves (Shdanow 1931, Sunderland 1965). Kuhlmann and colleagues analyzed the elimination route of macrophages from the murine sciatic nerve following transplantation. This study provided the finding that both local apoptosis and systemic elimination via lymphatic transport to local lymph nodes and the spleen are involved in this process (Kuhlmann et al. 2001). Volpi's group found that the perineurium of cadaveric sural nerves of patients suffering from polyneuropathy stained positive with D2-40, an antibody for podoplanin, which is a marker for lymphatic endothelium (Volpi et al. 2006, 2013; Hur et al. 2014; Bianchi et al. 2017). As previously mentioned, the perineurium is the mainstay of the blood–nerve barrier and is also completely impermeable to lymphatic fluid, making the endoneurial compartment particularly susceptible to increased pressure after direct trauma or other types of injury. It was therefore hypothesized that the lymphatic system may be involved in the so-called “miniature compartment syndrome” observable as a consequence of nerve injuries in which the perineurium is preserved, e.g., grades I–III according to Sunderland (Sunderland 1951; Lundborg et al. 1983; Frueh et al. 2020). Since no lymphatic vessels are located within the endoneurial compartment it was further speculated that this could easily result in obstructed capillaries due to increased local pressure (Frueh et al. 2020). Interestingly, intraneural pressures are seemingly about 2 mm HG higher than pressures in other tissues in general (Lundborg et al. 1983; Firrell 2019). It was hypothesized that the lymphatic vessels of a peripheral nerve are more likely obstructed by nerve compression than the intraneural vasculature, ascribing the former a crucial role in the pathophysiology of nerve compression syndromes. The lymphatic system might also play a pivotal role in the removal of “organic waste” following transection of the entire nerve in which also the lymphatic vessels have been severed. This was further supported by Meng et al., who found that the protein Prospero Homeobox 1 (Prox1), a marker for lymphatic endothelium, was significantly increased in murine sciatic nerves at 7 days after injury (Meng et al. 2020). Therefore, lymphangiogenesis, which is usually preceded by hemangiogenesis might likely be a crucial step for regeneration following peripheral nerve injury (Frueh et al. 2020) (Fig. 4).

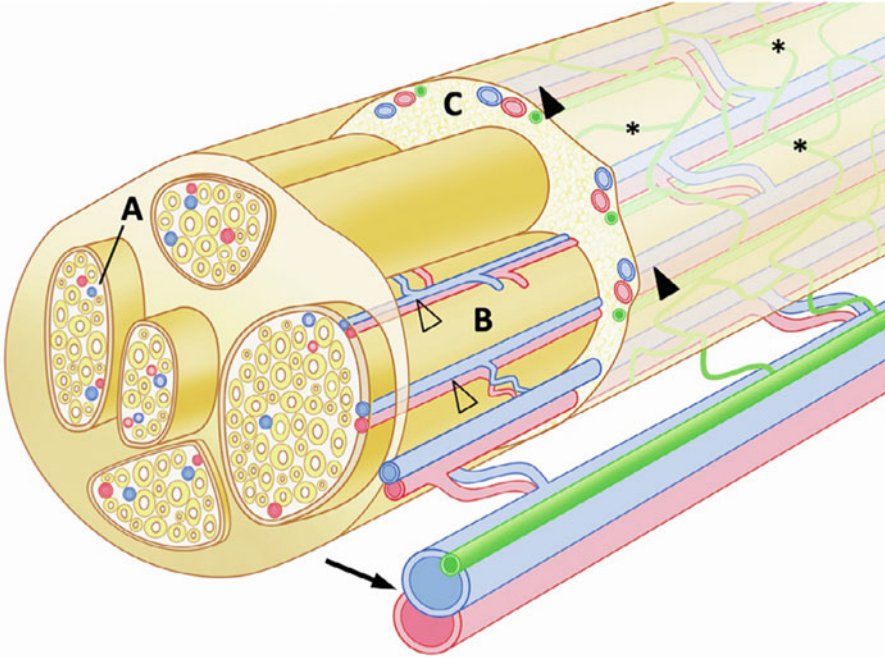


Fig. 4 The vascular and lymphatic system of peripheral nerves. Nerve fibers within fascicles are surrounded by the endoneurium (A), which contains a microvascular network. The perineurium (B) provides the vascularization to individual fascicles and is characterized by a small-caliber longitudinal vascular network (empty arrowheads). In contrast, the epineurial layer contains larger arteries and veins (C, black arrowheads) as well as a lymphatic vasculature (*). Larger-caliber and longitudinally oriented blood and lymphatic vessels (arrow) provide the macrovascular perfusion and hypothetically lymphatic drainage along the course of the nerve. (Adapted with permission from Frueh et al. (2020))

4 Physiology of Neural Microcirculation

Given the peripheral nerve function of signal transmission, it has been pointed out that its high metabolic activity undoubtedly plays a role regarding peripheral nerve vascularization. Despite this apparent relationship, no information has ever been published regarding the contribution of axonal transport via kinesin and dynein to these metabolic demands. The role of Schwann cells has also never been elucidated in this context (Muangsanit et al. 2018; Firrell 2019). Small molecules and oxygen are exchanged by diffusion between the capillaries and adjacent tissues, but this is limited by the critical distance of 100–200 μm (Saffari et al. 2020b). Oxygen can even directly pass from the epineurial into the endoneurial compartment under certain circumstances, e.g., exposure of the nerve during surgery, when higher atmospheric oxygen tensions are thought to drive nerve oxygen tension (Zochodne 2018). Capillaries within the endoneurial compartment are larger than those in other tissues, e.g., in

human median nerves the capillaries measure between 6 and 10 μm , which is larger than those in skeletal muscle (Olsson and Reese 1971; Bell and Weddell 1984b; Firrell 2019). However, for several reasons the peripheral nerve's axons are at risk of low oxygen supply. This relates to the fact that oxygen delivery to the endoneurial compartment is not only dependent on capillary flow although the latter plays a critical role in this regard (Lagerlund and Low 1987, 1993). On the one hand, capillary density is lower than in other tissues and the peripheral nerves' glia; e.g., Schwann cells, which are relatively densely packed, require additional blood supply (Low et al. 1989; Muangsanit et al. 2018). In the light of the aforementioned unknown oxygen demand of Schwann cells, this might result in low oxygen concentrations in relation to other tissues (Bell and Weddell 1984a; Muangsanit et al. 2018). On the other hand, the capability of autoregulation in response to changes in systemic blood pressure greatly differs between the extrinsic and intrinsic vascular system. While the extrinsic system can adapt to such variations by means of redistribution of blood supply via its extensive anastomosis (Lundborg 1970), the intrinsic system is unable to autoregulate, putting the endoneurial compartment at risk of severe tissue damage in case of falling blood pressure (Smith et al. 1977; Appenzeller et al. 1984; Zochodne 2002; Muangsanit et al. 2018). Despite this risk of low oxygen supply, the endoneurial compartment is well protected from ischemia in case of singular damage to one of its supplying vessels due to the arterioles, which supply the endoneurial plexus penetrating segmentally. This redundancy, together with the length to diameter ratio supported on the external vasculature (see above) allows for the safe "mobilization" of nerves during surgery; e.g., external neurolysis can be safely performed without severe impact on neural microcirculation (Graf et al. 1986). However, when internal neurolysis, e.g., interfascicular neurolysis, is performed this will likely have a severe impact on endoneurial blood flow due to disruption of the segmentally arranged transperineurial arterioles (Matsumoto 1983; Zochodne 2018).

4.1 Regulation of Neural Microcirculation

Endoneurial blood flow measured in rat models under normal conditions ranged between 15 and 20 ml/100 g/min (Low and Tuck 1984; Rundquist et al. 1985; Sladky et al. 1985), while estimates for the "whole nerve flow" ranged between 25 and 40 ml/100 g/min. Interestingly, this is lower than cerebral blood flow in humans, which is estimated as 40–80 ml/100 g/min (Fan et al. 2016; Zochodne 2018). Circulation within the nerve is both under control by the central nervous system and local metabolism-dependent regulation (Firrell 2019), whereas the latter, e.g., hypercarbia or acidosis, only affect epineurial microcirculation but not endoneurial blood flow (Low and Tuck 1984; Rechthand et al. 1988).

Experimental work in rodents has shown that peripheral nerves can tolerate low or even completely absent blood flow for time intervals between 60 and 180 min (Schmelzer et al. 1989) and permanent axonal damage occurs after at least 180 min of neural blood flow <5 ml/100 g/min (Zochodne 2018). Electrical stimulation of peripheral nerves results in increased oxygen consumption *ex vivo* (Gerard et al.

1927; Gerard 1927, 1930; Ritchie 1967) as well as increased nerve blood flow, additional recruitment of capillaries, and significant alterations of blood flow in the nerve roots and the spinal cord in vivo (Lundborg 1970; Takahashi et al. 1988; Firrell 2019). However, this is probably linked to direct vasodilation mediated by the peptidergic nerve fibers, which innervate the vasa nervorum rather than by a direct metabolic response (Zochodne and Ho 1991b; Zochodne 2018).

While the epineurial blood vessels receive their innervation by adrenergic, peptidergic, and serotonergic nerve fibers of the autonomic nervous system, the perineurial vessels are innervated by adrenergic fibers (Appenzeller et al. 1984; Rechthand et al. 1986; Koistinaho et al. 1991; Dhital and Appenzeller 1998; Zochodne 2018; Firrell 2019). The unmyelinated nerve terminals (Hromada 1963), which innervate the vasa nervorum, are referred to as the “nervi nervorum” and arise from the peripheral nerve itself, which therefore “auto-innervates” its vessels (Rechthand et al. 1986). In particular, the fine autonomic and sensory nerve fibers exit the endoneurial compartment and travel toward the epineurium innervating local arterioles. Infusion of norepinephrine into the arteries supplying a peripheral nerve (Selander et al. 1985), topical application of norepinephrine (Zochodne and Low 1990; Kihara and Low 1990), or direct stimulation of the sympathetic system (Lundborg 1968) under otherwise normal physiological conditions cause a strong vasoconstrictive response with a significant decrement in neural blood flow. Conversely, chemical sympathectomy via the application of guanethidine (Zochodne et al. 1989, 1990) results in enhanced endoneurial perfusion by lowering the microvascular resistance (Firrell 2019). Interestingly, vasoconstriction in consequence to application of sympathomimetic agents displays a heterogeneous and segmental pattern, implicating the presence of different zones of vasoreactivity, which could be thought of as some kind of precapillary “gatekeepers” (Zochodne and Low 1990; Zochodne 2018). As mentioned previously, innervation of epineurial vessels is also mediated by peptidergic sensory nervi nervorum, which in contrast to their adrenergic counterparts provide vasodilatory input, mediated by transmitters such as calcitonin gene-related peptide (CGRP) and substance P (Appenzeller et al. 1984; Dhital and Appenzeller 1998). Therefore, exposure of the epineurium to capsaicin results in depolarization of the peptidergic terminals, causing a strong increase in neural blood flow. Conversely, application of CGRP or substance P antagonist blocks the aforementioned hyperemia (Zochodne and Ho 1991a). The serotonergic fibers innervating the epineurial vessels exert the same effects as the peptidergic fibers, e.g., serotonin is a mediator of vasodilatation.

Besides adrenergic, serotonergic and peptidergic mediation, neural blood flow, and especially endoneurial microcirculation can be modulated by several other molecules. While acetylcholine (Thomsen et al. 2000), nitric oxide (Zochodne et al. 1999), and prostaglandin (Kihara and Low 1995) act as effectors of vasodilatation, vasoconstriction is a result of the application of vasopressin, neuropeptide Y, endothelin (Zochodne et al. 1992), or angiotensin II (Kihara et al. 2000; Yorek 2015; Zochodne 2018).

Although it is reasonable to believe that the aforementioned physiological pathways apply to all peripheral nerves, this hypothesis has never been tested in the form of a comprehensive investigation (Zochodne 2018).

5 Pathophysiological Aspects of Neural Microcirculation

The role of the vasa nervorum and neural microcirculation in peripheral nerve injuries still remains a subject of scientific debate. In the light of the ongoing development and improvement of techniques to measure neural blood flow, several previously established paradigms are now the subject of in-depth scientific reevaluation (Zochodne 2018). Among these paradigms are the assumptions that focal nerve trauma results in ischemia of the respective nerve trunk (Lundborg 1975) and that nerve compression syndromes cause secondary ischemia due to compromised venous blood flow. However, there is growing evidence that these assumptions are not generally applicable (Zochodne 2018).

In contrast to the central nervous system, e.g., the brain, peripheral nerves are relatively well protected from ischemia, and therefore fairly drastic measures are required if ischemic nerve damage is to be induced experimentally. Such approaches including vessel embolization with microspheres (Nukada and Dyck 1984, 1986; Nukada et al. 1985; Kihara et al. 1993), vascular ligation (Sladky et al. 1985; Nukada and Dyck 1986; McManis and Low 1988), or topical application of the aforementioned vasoconstrictor endothelin (Zochodne et al. 1992) provided the insight that an overall reduction of neural blood flow to 5 mg/100 g/min or lower for a least 180 min is required to damage axons permanently (Zochodne 2018). In cases where the ischemic interval lasts between 60 and 180 min, ischemia-reperfusion injury might hinder full functional recovery even when circulation has been restored completely (Schmelzer et al. 1989). For instance, in limbs with peripheral arterial occlusive disease, focal ischemic neuropathy can be the result of compromised vascular patency (Nukada et al. 1996). In animal models, induction of limb ischemia resulted in degeneration of the central aspects of nerves with compromised blood supply (Adams 1943; Hess et al. 1979), similar to findings after transplantation of a non-vascularized ulnar nerve (Slutsky 2013). The common practice to use a tourniquet to perform surgery under ischemic conditions does logically also affect the peripheral nerves. These disturbances can be transient or even permanent, but it remains to be elucidated whether they are caused directly by the mechanical pressure exerted by the tourniquet or result from compromised neural microcirculation (Ochoa et al. 1972; Zochodne 2018; Firrell 2019).

5.1 Nerve Compression

Although some authors speculate that compromised neural microcirculation or ischemia, respectively, is one of the key elements involved in nerve compression pathology, these findings have been reported rather circumstantially and it must be noted that there is limited evidence to support this hypothesis (Zochodne 2018; Firrell 2019). As described previously, ischemia is not easily induced in peripheral nerve trunks and experimental models usually require relatively drastic measures to cause limb or nerve ischemia (Zochodne 2018). Especially in the light of the extensive anastomoses between the extrinsic and intrinsic vascular system, doubts

have been formulated that restricted sites of nerve compression or partial nerve injuries might induce ischemia of the whole nerve. Instead of ischemic sequelae, pathological findings in patients with compression neuropathy might be directly related to mechanical aspects of nerve compression, e.g., invagination of axons (Ochoa et al. 1972; Zochodne and Ho 1990; Dyck et al. 1990; Zochodne 2018). Noteworthy and in contrast to the observable degeneration of the nerve's central aspects as a result of generally compromised vascular patency (Adams 1943, Hess et al. 1979), animal experiments indicate that compression predominantly affects the nerve's peripheral parts (Mackinnon et al. 1984; Mackinnon and Dellon 1986; O'Brien et al. 1987; Firrell 2019). Ongoing and extensive nerve compression will eventually result in a decrease of neural blood flow, resulting in permanent axonal damage and cell death (Mackinnon et al. 1984; Zochodne 2018). Still, it remains to be elucidated if this decrement in neural blood flow is related to the aforementioned "miniature compartment syndrome" of the endoneurial space as was suggested by Goran Lundborg and colleagues (1983) or if instead endoneurial ischemia is a result of increased endoneurial intercapillary distance (Low et al. 1980; McManis et al. 1986; Zochodne 2018). Interestingly, measurable changes in blood flow might be preceded by increased vascular permeability (Sonabend et al. 2012).

5.2 Nerve Crush

Experimental work showed that neural blood flow in the respective nerve segment was not decreased 2 h following focal nerve crush nor was there any evidence for the aforementioned phenomenon of ischemia-reperfusion injury. Instead a marked increment of neural blood flow in the crushed segment was observable, exceeding both the flow in the proximal stump and uninjured nerves (Zochodne and Ho 1990, 1992a; Xu et al. 2010; Zochodne 2018). Even extended periods of crushing, e.g., 120–180 min, do not seem to cause significant decrements in posttraumatic neural blood flow in the respective segment. Additionally there was no evidence for ischemia of the segments proximal and distal to the crush site (Zochodne and Ho 1992a). Increments of neural blood flow could be observed for 1–2 days following crush injury and were traceable for up to 2 weeks. Interestingly, hyperemia was observable in both the extrinsic (epineurial) and intrinsic (endoneurial) vascular system. The underlying reasons for these findings remain to be elucidated in detail but are thought of as a sort of compensatory mechanism to prevent nerve ischemia following crush injury involving segments of considerable length. In the case of the endoneurial compartment, dilatation of microvessels has been identified as one of the driving forces behind the developing hyperemia (Podhajsky and Myers 1993; Zochodne 2018).

5.3 Nerve Transection

Similar to the findings following nerve crush, nerve transection in animal models also does not result in ischemia but in marked hyperemia, both in the proximal and distal nerve segment. Experimental nerve grafting provided the finding that even in

case of a nonvascularized nerve graft, neural blood flow was preserved even 7 days after grafting (Shupeck et al. 1989; Zochodne 2018).

The observed hyperemia seems to be mediated by CGRP and nitric oxide (NO), released from axonal terminals in the injured area of the nerve. CGRP, besides its aforementioned vasodilatory effect seemingly also plays a role in pain signaling as well as proliferation of Schwann cells. NO is synthesized by the enzyme endothelial nitric oxide synthetase (eNOS), which is highly accumulated in axonal terminals (Zochodne 2018). Neural blood flow is therefore increased in the early phase following nerve injury due to microvessel dilatation as described above (Podhajsky and Myers 1993; Zochodne and Nguyen 1997; Zochodne et al. 1999). Increased blood flow is maintained for up to a month following nerve transection, but besides the aforementioned mediators, other mechanisms such as angiogenesis are involved at later time points (Höke et al. 2001; Zochodne 2018). Interestingly, this 4-week time frame corresponds very well to the observed formation of a “fibrin cable” between the severed nerve stumps, which acts as a sort of guidance scaffold for regenerating axons (Höke et al. 2001; Kaplan et al. 2015; Heinzel et al. 2020). Other experimental work provided the insight that following chronic neurotmesis, and therefore in the absence of any viable axons, neural blood flow declines to ischemic levels (Höke et al. 2001), which might hinder regeneration at this late time point after injury. Given the release of both aforementioned transmitters from axonal endbulbs, this might explain the onset of ischemia in cases of complete absence of viable axons. There is no conclusive evidence yet that the observable decline of regenerative potential after time intervals exceeding 12–18 months following nerve injury (Gordon et al. 2011) is directly related to the aforementioned ischemic conditions (Zochodne 2018). Other studies provided evidence that poor or hindered regeneration might also be related to Schwann cell atrophy (Ronchi et al. 2017), synaptic failure (Sakuma et al. 2016), or alterations of the respective target organs (Campbell 2008).

Noteworthy, marked hyperemia as observable in cases of focal nerve compression, crush, or transection injury is not a regular finding in all types of nerve injuries. Notable exceptions are chronic constriction injuries, tumor strangulations, compartment syndromes, and stretch injuries (Zochodne 2018).

5.4 Chronic Constriction Injury

Chronic nerve constriction injury is a well-established model of neuropathic pain, induced by placing ligatures around the nerve (O'Brien et al. 1987; Bennett and Xie 1988; Chen et al. 2020). The resulting swelling and eventual strangulation eventually lead to ischemia of the nerve in addition to the inflicted mechanical stress. In consequence, the respective axons, especially large caliber fibers, are heavily damaged (Sommer and Myers 1996; Levy and Zochodne 1998; Zochodne 2018).

5.5 Nerve Stretching

Given the aforementioned perpendicular course of the transperineurial vessels and their susceptibility to kinking, the deleterious effect of nerve stretching on intraneural

microcirculation is underpinned. While venous blood flow decreases with an 8% strain, arterial and capillary circulation is heavily compromised with strain of about 10%. Complete circulatory arrest can be observed when strain reaches 15% (Stanton-Hicks 2018). Notably, it remains unknown whether the extrinsic or intrinsic vascular system is primarily affected by nerve stretching or if it is a combination of both (Firrell 2019). Regarding clinical application of this knowledge, the degree of tension at a site of nerve repair can be directly linked to intraneural microcirculation. While Millesi advised that *any tension* must be avoided at the repair site (Millesi 1982) others including peripheral nerve surgery pioneer Herbert Seddon opted for a moderate amount of stretching to be tolerable, proposing at least 6% of “normal nerve elasticity” (Seddon 1975). Sunderland insinuated that manipulation of the proximal and distal segments of a nerve to achieve an end-to-end connection should not exceed the critical size limit of 8 cm (Sunderland 1991; Stanton-Hicks 2018). Although knowledge regarding vascular anatomy and intraneural microcirculation has increased, the topic still remains a matter of fiery scientific debates (Mahan 2019).

5.6 Neuroma

Neural blood flow could also be directly related to axonal growth and neuroma formation. In chronic neuromas, heterogeneous structures of unorganized axonal sprouts, and connective tissues, marked variations of intraneural blood flow are observable. While zones of increased circulation feature areas of observable angiogenesis and axonal minifascicles, areas mainly consisting of connective tissue without axons are ischemic and avascular (Zochodne et al. 1995; Zochodne and Nguyen 1997; Xu and Zochodne 2002; Zochodne 2018). In the case of the common peroneal nerve which usually shows only little recovery following trauma, the blood supply and therefore neural microcirculation was speculated to be one of the main factors responsible for poor outcomes after injury (Garozzo et al. 2004; Kadiyala et al. 2005; Ugrenovic et al. 2013).

5.7 Neuropathies

In various peripheral neuropathies and chronic neuropathic pain there are structural, functional, or molecular alterations at the blood–nerve interface (Ubogu 2020). Lim et al. found that blood–nerve barrier dysfunction contributes to the generation of neuropathic pain (Lim et al. 2014) and peripheral nerve autoimmune disorders, such as Guillain-Barre syndrome and chronic inflammatory demyelinating polyradiculoneuropathy have been pathologically associated with heightened permeability of the blood–nerve interface (Ubogu 2020).

5.8 Diabetic Neuropathy

Endoneurial microvessel wall thickening has been described in association with chronic peripheral neuropathies such as diabetes mellitus (DM) and peripheral nerve

vasculitis (Bosetti et al. 2016), but the possible involvement of neural microcirculation in DM is a controversial subject of scientific debate (Zochodne 2002). The incidence of diabetic neuropathy has been linked to vascular complications arising from DM in human patients and pathological changes of the vasa nervorum, e.g., microthrombosis and destruction of epineurial vessels have been identified in human patients suffering from DM or DPN (Dyck et al. 1985, 1986; Korthals et al. 1988; Giannini and Dyck 1995; Dyck and Giannini 1996; Malik 2014; Zochodne 2018). Interestingly, experimental investigation in rodents revealed that DM is associated with defects of lymphatic vessels, supporting the aforementioned hypothesis of lymphatic involvement in peripheral nerve disease (Scallan et al. 2015; Frueh et al. 2020). Supporters of the “impaired neural blood flow” theory have therefore suggested hypoxic or ischemic conditions as main drivers of diabetic neuropathy (Dyck 1989; Zochodne 2018). This is further supported by the plethora of experimental approaches by which neural function is supported via improvement of vascular function (Coppey et al. 2001, 2006; Yorek 2015; Kobayashi and Zochodne 2018; Zochodne 2018). Opponents of the “impaired neural blood flow” theory argue that it is not the circulation-enhancing effect of the respective therapies, but these therapies’ direct effects on the nerve that improve nerve function and therefore symptoms of the underlying disease (Zochodne 2018; Kobayashi and Zochodne 2018). Additionally, neither changes in neural blood flow, in general, nor endoneurial blood flow, in particular, could be detected in experimental models using different methodology (Pugliese et al. 1989; Zochodne and Ho 1992b, c; Tilton et al. 1995; Chang et al. 1997; Zochodne and Nguyen 1999; Zochodne 2018). Instead, endoneurial blood flow has been shown to be normal in experimental models putting the aforementioned hypothesis into question (Zochodne 2018). Another reasonable point of criticism relates to the fact, that it is rather unlikely that a general decrease in neural blood flow would selectively damage sensory axons, but leave their motor counterparts unharmed (Zochodne 2018). It was therefore suggested that instead of microvascular dysfunction, compromised oxygen extraction, oxidative stress, and axonal dysfunction are the main pathophysiological mechanism involved in the development of diabetic neuropathy, triggering dysfunction of capillaries within the affected peripheral nerves. In this context, reactive hyperemia in the early phase of the disease is considered a sequela of the aforementioned capillary dysfunction rather than its underlying reason. Although there is clear evidence that DM targets the vasa nervorum and affects vasodilatation of arteries and arterioles, it remains to be elucidated whether microvascular changes trigger the development of diabetic neuropathy or just progress in parallel with the pathological changes affecting neurons and their respective axons.

6 Role of Neural Vascularization and Microcirculation in Peripheral Nerve Regeneration

Hemangiogenesis and adequate blood supply are key steps for peripheral nerve regeneration following injury. The spatiotemporal course of blood vessel formation, Schwann cell migration, and axonal regeneration is a meticulously orchestrated

process in which vascularization is the crucial prerequisite for nerve regeneration to occur (Hobson et al. 1997, 2000; Hobson 2002; Muangsanit et al. 2018; Saffari et al. 2020b, 2021). Regenerating axons require Schwann cells, aligned in the columnar arrays called the “Bands of Büngner,” for directed growth in the direction of the distal nerve stump (Jessen and Mirsky 2016). Schwann cell migration in turn follows vascular sprouting, underpinning the important interaction between neo-vascularization, Schwann cell migration and alignment and axonal regeneration (Muangsanit et al. 2018). The aforementioned vasophilic migration of neural cells is therefore heavily dependent on both vascularization and neural microcirculation during the phase of nerve regeneration (Caillaud et al. 2019). The rate of vascularization as well as the blood vessel caliber was shown to correlate with axonal regeneration; therefore axonal regeneration is limited in cases of decreased blood vessel density and/or caliber (Weddell 1942; Tarlov and Epstein 1945).

Successful regeneration is highly dependent on capillary function, including both their numbers and permeability (Nukada 1988; Weerasuriya 1988, 1990; Mizisin and Weerasuriya 2011). While it was hypothesized that increased numbers and permeability of capillary vessels facilitates disposal of organic waste (Hobson et al. 1997; Muangsanit et al. 2018), this might also heavily depend on lymphangiogenesis and lymphatic vessel function (Frueh et al. 2020).

Despite the growing body of literature and knowledge regarding the interplay of vascularization and nerve regeneration, studies elucidating the exact spatiotemporal course of these mechanisms during nerve regrowth are still sparse (Caillaud et al. 2019).

Caillaud et al. injected glycerol in rat sciatic nerves (Vallat et al. 1988) and evaluated vascular regrowth until 60 days after injury by means of electron microscopy. Two days after glycerol injection they found that the preexisting blood vessels were ruptured and erythrocytes as well as platelets had extravasated. Interestingly, neoformation of capillaries was already observable at this early time point. One week after injection injury, fibroblast migration and abundant collagen formation was observable, with the fibroblasts later differentiating into perineurial cells. Thus, these findings indicate angiogenesis as well as formation and maturation of a newly formed blood–nerve barrier between 2 and 3 weeks after injection injury. Complete regeneration of the intrinsic vascular system was observable in the aforementioned study 60 days after nerve injury (Caillaud et al. 2019).

Podhajsky and Myers studied the vascular response after crush (Podhajsky and Myers 1993) and transection (Podhajsky and Myers 1994) of the sciatic nerve in rats and reported two distinct phases following the aforementioned injuries. During the early phase, which included the first 7 days following nerve injury, an increase in blood vessel size was observable whereas the number of blood vessels was not altered compared to pre-injury. This was followed by an increased number of blood vessels during the second phase, which comprised the second to sixth week following injury. While the first phase encompasses the recruitment of macrophages and removal of organic waste in the wake of the breakdown of axons and myelin sheaths during Wallerian degeneration, the second phase comprised cellular proliferation, axonal regrowth, and formation of myelin sheaths (Podhajsky and Myers 1993, 1994; Caillaud et al. 2019).

In case of segmental nerve injuries, which require interposition of an autologous nerve graft, a decellularized allograft, or a nerve guidance conduit, vascularization is of crucial importance to maintain oxygen supply and prevent necrosis of central parts of the regenerating nerve (Muangsanit et al. 2018). This is particularly important in case of large diameter nerve grafts, considering the maximum diffusion distance of oxygen of around 100–200 μm (Jain et al. 2005; Rouwkema et al. 2010; Saffari et al. 2020b). Since transplanted nerves are cut off from the vascular supply, they require reestablishment of their blood supply by means of angiogenesis (Bassilios Habre et al. 2018). Angiogenesis refers to the remodeling of preexisting vessels, formed during embryonic development in the process of vasculogenesis, into new areas, e.g., the transplanted nerve or nerve guidance conduit (Akhavani et al. 2008; Kolte et al. 2016). It has been established that revascularization of an autologous nerve graft is accomplished by longitudinal inosculation, e.g., ingrowth of host vessels from both stumps which then form anastomoses with the preexisting donor vessels within the autograft (Lind and Wood 1986; Penkert et al. 1988; Best et al. 1999a, b; Saffari et al. 2020b). If, however, the metabolic demands of the nerve surpass the capacity, which can be provided by the inosculated blood vessels, centripetal revascularization, ingrowth of blood vessels from the surrounding tissue bed, occurs (Tarlov and Epstein 1945; Terzis et al. 1995; Saffari et al. 2020b). With the help of μCT visualization techniques, Saffari et al. provided the information that inosculation occurs predominantly from the proximal nerve stump (Saffari et al. 2020c). This finding confirmed a theory suggested by Chalfoun et al. (2003) and abandoned the previous assumption that the mechanism of inosculation would involve both the proximal and distal nerve stump equally (Saffari et al. 2020b). These considerations also support the finding that graft failure due to necrosis of its mid-section is proportional to the nerve graft's length (Terzis et al. 1995; Lovati et al. 2018; Saffari et al. 2020b, c) as well as its diameter (Breidenbach and Terzis 1987).

While it is established that vascularization is the paramount prerequisite for vasophilic migration of glial cells and axonal sprouts, the exact mechanisms regulating these finely orchestrated processes remain to be elucidated in detail (Ferretti et al. 2003; Merolli et al. 2009; Goedee et al. 2013; Caillaud et al. 2019). Caillaud et al. concluded that the vascular system's initial response to trauma is an increase of blood vessel size, mediated by substance P, CGRP, and others. The ensuing vasodilatation is associated with the release of monocyte chemoattractant protein-1 by Schwann cells, resulting in recruitment of macrophages. Macrophages then secrete VEGF, which in turn triggers hemoangiogenesis, and the newly formed vessels act as scaffold for migrating Schwann cells. The Schwann cells then align in column-like Bands of Büngner, which facilitate axonal regrowth and therefore nerve regeneration (Caillaud et al. 2019).

Cattin and colleagues suggested that the driving force beyond Schwann cell migration and axonal regeneration are vascular endothelial cells and that they do so by secreting the aforementioned VEGF, which stimulates angiogenesis (Cattin et al. 2015) as well as Schwann cell migration (Muratori et al. 2018) and induces accelerated axonal regeneration from dorsal root ganglia (Sondell et al. 1999; Hobson et al. 2000; Wongtrakul et al. 2002; Mohammadi et al. 2013; Lee et al.

2016; Schlau et al. 2018; Nishida et al. 2018; Saffari et al. 2020b). Interestingly, Schwann cells in turn promote endothelial cell migration in vitro, which again emphasizes the importance of neurovascular congruence because steady advancement of the “regenerative front line” can be sustained through these interactions (Ramos et al. 2015).

7 Studying Neural Vascularity and Microcirculation

Identification of neural vessels and measurements of neural microcirculation is of particular interest both from a scientific as well as a clinical point of view.

Especially during deep tissue dissection, it can be challenging to distinguish nerves from the surrounding tissue, which increases the risk of nerve injury. To identify the nerve and its vasa nervorum, intraoperatively administered fluorescent indocyanine green (ICG) can be used as a guide. Intravenously injected ICG distributes systemically and fills the lumen of the epineural vessels. Visualization of the nerve pathways and Vasa Nervorum could be displayed in real-time with a near-infrared camera (Kwon et al. 2019).

Neural blood flow measurements can be performed via Laser Doppler flowmetry (LDF) as was demonstrated by Seiler and colleagues who used this approach to study median nerve blood flow during carpal tunnel release (Seiler et al. 1989). As LDF allow for detection of rapid alterations in blood flow it is advantageous for guiding the surgeon intraoperatively when manipulating the nerve (Firrell 2019). Theriault et al. applied LDF measurements in diabetic patients undergoing sural nerve biopsies (Theriault et al. 1997). However as pointed out by Zochodne, these measurements are often focused on the epineurial circulation, avoiding measurements over shunt vessels, arterioles, and venules (Zochodne 2018).

Although it seems that interest in measuring neural blood flow in human patients has declined within the last 10–20 years, new developments, e.g., neuromuscular ultrasound, have somehow renewed the overall diminished attention (Zochodne 2018). Despite the limitation that ultrasound probes cannot be placed directly adjacent to the respective nerve of interest, except when used in the operation room, the method has been used in several clinical investigations, most of them focusing on the median nerve in carpal tunnel syndrome (Reynolds et al. 2004; Joy et al. 2011; Evans et al. 2012; Kutlar et al. 2017; Borire et al. 2017; Zochodne 2018).

Imaging of the peripheral nervous systems by means of Magnetic Resonance Imaging (MRI) and Computer Tomography (CT) has made a huge leap forward in recent years (Stoll et al. 2013; Ohana et al. 2014; Chhabra 2015; Holzgrefe et al. 2019) and some preclinical (Heimel et al. 2019; Thompson et al. 2020) and clinical studies (Bäumer et al. 2014; Yan et al. 2019) addressing this topic have already been published. It is therefore likely that sooner or later these methods, enhanced with contrast agents, will allow both for high-resolution visualization of the vasa nervorum as well as precise measurements of neural blood flow (Zochodne 2018).

8 Clinical Applications to Improve Vascularization and Microcirculation of Peripheral Nerves

The improved understanding of the vasa nervorum directly influences clinical practices in reconstructive surgery, especially in covering nerve defects and transplanting nerves. Commercially available nerve conduits have shown considerable success in transplants covering a short gap (Ray and Mackinnon 2010; Isaacs and Browne 2014), but unfortunately, this result could not be reproduced with long nerve lesions (Kornfeld et al. 2019). Autograft or allograft repair of such defects is mandatory, but with longer grafts the risk for central necrosis due to insufficient blood supply is strikingly increased. Conduits on the other hand, commonly do not contain living cells or vasculature, putting regenerating axons and the surrounding Schwann cells at risk for necrosis. In case of hampered blood supply from the surrounding tissue, e.g., a scarred wound bed, vascularization strategies are also mandatory to ensure cellular survival and to prevent central necrosis. Vascularization is therefore a major challenge for surgeons and tissue-engineers striving to develop patient-specific substitutes for peripheral nerve reconstruction (Muangsanit et al. 2018; Saffari et al. 2020b).

According to Muangsanit et al. vascularization strategies for peripheral nerve repair comprise five main groups: (1) Vascularized nerve grafts, (2) Nerve grafts with implanted vessels, (3) Nerve guidance conduits with implanted vessels, (4) Biogenic vascularized nerve conduits, and (5) Engineered neural tissue (Muangsanit et al. 2018).

8.1 Vascularized Nerve Grafts

Use of vascularized nerve grafts (VNGs) has been suggested as superior to non-vascularized nerve grafts (NVGs) since 1976 when Taylor and Ham used a free, vascularized segment of the radial nerve to bridge a segmental defect of the contralateral median nerve in the same patient (Taylor and Ham 1976). Almost 30 years earlier Strange used a pedicled ulnar nerve graft for reconstruction of the median nerve (Strange 1947). Selection of a potential donor for a VNG depends on the vascular supply pattern of the respective nerve (Breidenbach and Terzis 1984, 1987; El-Barrany et al. 1999). Breidenbach and Terzis suggested that a nerve that receives blood supply from one dominant arterial pedicle should be considered especially favorable to be used as a VNG since it can easily be transferred based on the one dominant vascular system (Breidenbach and Terzis 1987). El-Barrany et al. consented to the aforementioned authors' suggestions, but stated that also nerves with multiple pedicles could favorably be used as potential VNGs (El-Barrany et al. 1999).

As mentioned previously, neovascularization of a nerve transplant can be observed within the first week following peripheral nerve grafting, usually starting from the third postoperative day (Mani et al. 1992; Muangsanit et al. 2018). If neovascularization is therefore accelerated by means of a VNG, this will facilitate

nerve regeneration (Mani et al. 1992; Bertelli et al. 1996; Donzelli et al. 2016; Muangsanit et al. 2018; Saffari et al. 2020b).

The underlying reasons for improved nerve regeneration due to vascularization are thought to be (1) an increased number of Schwann cells, (2) minimized intraneural fibrosis due to avoidance of early ischemia as well as decreased fibroblast infiltration, and (3) enhanced axonal regeneration (Breidenbach and Terzis 1987; Oudega et al. 2001; Donzelli et al. 2016; Muangsanit et al. 2018; Saffari et al. 2020b). Although increased myelination of axons was reported after use of a VNG in an animal model featuring a scarred, nonvascularized wound bed, conduction velocity was not significantly altered in other experimental studies (Koshima and Harii 1985; Mani et al. 1992; Ozcan et al. 1993a; Saffari et al. 2020b).

In case of an unscarred, well-vascularized wound-bed, results of nerve reconstruction with VNGs vs NVNGs are ambivalent. While no differences were observable in several rat model studies (McCullough et al. 1984; Pho et al. 1985; Seckel et al. 1986), rabbit model studies reported superior results after nerve reconstruction with VNGs (Restrepo et al. 1985; Shibata et al. 1988; Kanaya et al. 1992). On the contrary, Daly and Wood found increased blood flow in the NVG group after nerve grafting in a canine model (Daly and Wood 1985). It was hypothesized that these conflicting results might be related to the length of the nerve gap and choice of the animal model. Rat models, although advantageous in regard to their use as a “bioassay” (Saffari et al. 2020b), have a highly limited translatability due to markedly accelerated nerve regeneration and profound differences regarding what is considered a “critical size nerve defect” (see chapter ► “Fibrin in Nerve Tissue Engineering” of this book) (Kaplan et al. 2015; Saffari et al. 2020b).

Apart from experimental evaluations in animal models, several studies have investigated the use of NGVs vs NVGs in the clinical setting, almost all of them were case reports or case series (Farzan et al. 1970; Doi et al. 1987, 1992; Rose et al. 1989; del Pinal et al. 2007; Bertelli and Ghizoni 2007; Terzis and Kostopoulos 2009, 2010a, b; D’Arpa et al. 2015). In accordance with the ambiguous results reported in experimental studies, clinical applications also produced contrary results, with some authors also advising against use of VGNs due to the risk of vessel thrombosis and resulting graft necrosis (Merle and Dautel 1991; Saffari et al. 2020b).

Although there is still no consensus regarding the feasibility of VNGs for reconstruction of segmental nerve defects, Saffari et al. extrapolated the following recommendations regarding their use from the aforementioned case reports and case series (Saffari et al. 2020b): Use of a VNG should be considered in the case of (1) a large nerve gap exceeding 6–7 cm, (2) a large diameter recipient nerve, (3) a scarred badly or even nonvascularized wound bed with little or no chance of sufficient centripetal revascularization of the nerve graft, and (4) a significant delay between the date of the nerve injury and the date of surgical treatment, exceeding a period of 2 years (Koshima and Harii 1985; Restrepo et al. 1985; Shibata et al. 1988; Kallio and Vastamaki 1993; Terzis and Kostopoulos 2010b; D’Arpa et al. 2015). As noted by Saffari and colleagues, randomized controlled clinical trials, including patients with homogeneous nerve injuries, surgical treatments, and comparable follow-up time are mandatory to elucidate the value of VNGs to reconstruct segmental nerve

defects. Additionally, the aforementioned authors advised the use of quantitative assessment tools such as ultrasound (Arányi et al. 2018) to reflect neovascularization and blood flow. In contrast to these suggestions, clinical studies predominantly rely on the Tinel sign, electrophysiological evaluations, and assessment of motor and sensory function as surrogate parameters for improved nerve graft vascularization (Saffari et al. 2020b).

Based on El-Barrany's classification system (El-Barrany et al. 1999) and their acceptable donor site morbidity, the sural and saphenous nerve are used most frequently as donors for VNGs (Staniforth and Fisher 1978; Breidenbach and Terzis 1984; Terzis et al. 1995; Muangsanit et al. 2018; Saffari et al. 2020b). Successful use of other nerves such as (1) the radial nerve including its superficial branch or even the posterior interosseus nerve, (2) the ulnar nerve, or (3) the superficial peroneal nerve has been demonstrated (Taylor and Ham 1976; Townsend and Taylor 1984; Hattori et al. 2005; Shafi et al. 2010; D'Arpa et al. 2015; Foo et al. 2019). However, regarding the aforementioned nerves' considerable donor site morbidity, their use is preserved for special indications such as a particularly large nerve gap or the requirement to reconstruct multiple concomitant nerve injuries (Muangsanit et al. 2018; Saffari et al. 2020b). The advantages of using a VNG are opposed by the need for microsurgical experience and technique when anastomosing the respective vessels. Additionally, the resulting donor site morbidity must be carefully reflected and balanced against the expected functional gain for each respective patient. Thus, alternatives such as artificial vascularized nerve grafts, vascularized scaffolds, or bioengineered neural tissues are emerging as potential valid alternatives, evading the donor site morbidity, which accompanies the harvesting of VNGs (Muangsanit et al. 2018).

8.2 Nerve Guidance Conduits with Implanted Vasculature (See Chapter ► "Biomaterials and Scaffolds for Repair of the Peripheral Nervous System" of This Book)

Implantation of blood vessels inside a nerve conduit was pioneered by Kakinoki in 1997, who included sural vessels of rats inside a hollow silicone conduit. Unfortunately, no nonvascularized control group was used, although nerve regeneration did reportedly occur across a 25 mm defect of the sciatic nerve (Kakinoki et al. 1997). Kosaka and colleagues used a subcutaneous artery, which they also inserted into a silicone tube. In comparison to a nonvascularized tube, microvasculature within the regenerating nerve was significantly greater while both morphological and functional recovery were also enhanced (Kosaka 1990). However, early studies evaluating this approach almost exclusively made use of silicone tubes, which bare the disadvantages of non-biodegradability, potential compression of adjacent tissues, and decreased range of motion of neighboring joints, and may cause synovitis (Lanzetta et al. 1994; Muangsanit et al. 2018). Despite these disadvantageous features of silicone nerve conduits, Luo's group used silicone conduits including a harvested subcutaneous artery in a clinical study. The authors reconstructed one

radial nerve defect and five defects of the median and ulnar nerve, respectively, in a total of seven patients, ranging from 3 to 5 cm in length. The authors reported excellent and good results in 77.8% of cases. Interestingly, at the time when the silicone was removed surgically, the implanted artery could not be identified anymore (Luo and Wang 1992).

Kaizawa et al. made use of a l-lactide/ ϵ -caprolactone nerve guidance conduit, vascularized by means of ulnar artery implantation and seeded with undifferentiated bone marrow stromal cells to reconstruct ulnar nerve defects in dogs. Nerve regeneration through these cell-loaded conduits was inferior compared to the autograft control group, although these differences were not statistically significant (Kaizawa et al. 2016). One year later, the same group published the results of their attempt to bridge a 20-mm defect of the sciatic nerve in rats, using a silicone tube vascularized by sural vessel implantation and substituted with either a thermally decellularized allogenic nerve matrix or mesenchymal stem cells. Although there was no comparison with autografts regarding histomorphometric or electrophysiological parameters, the vascularized conduits were compared to autologous nerve grafts in regard to revascularization and proved superior up to 4 weeks following nerve repair (Kaizawa et al. 2017).

8.3 Generation of Vascularized Nerve Grafts and Conduits via Arteriovenous Shunting

The procedure to vascularize tissues by implanting them in close proximity to an arteriovenous fistula or loop (AV loop) is a well-established approach, which has been applied to skin (Falco et al. 1992), bone (Horch et al. 2014), and composite tissues, e.g., free flaps (Henn et al. 2020a, b), prior to their transplantation both in preclinical as well as clinical studies. This technique has also been tested regarding generation of vascularized nerve grafts (Cavadas and Vera-Sempere 1994; Saray et al. 2002) or nerve conduits (Ozcan et al. 1993b; Iijima et al. 2016). However, nerve regeneration in comparison to an “original” VNG was only seldomly assessed in these studies. Additionally, one has to consider the disadvantages of this approach, e.g., two-staged surgery and prolonged denervation time of the target organs (Muangsanit et al. 2018). In conclusion, generation of vascularized nerve grafts by means of implantation in close proximity to an AV loop is a valuable addition to the armamentarium of peripheral nerve surgeons. However, given the steadily advancing techniques and possibilities in the field of tissue engineering, other approaches, such as vascularized biogenic conduits will likely overtake them in the long run.

8.4 Generation of Vascularized Biogenic Conduits

After placing an artificial conduit, e.g., a silicone tube, in close proximity of a blood vessel a vascularized pseudosheath will develop (Gu et al. 2011). As was elucidated in a primate model, this pseudosheath is composed of three main layers: (1) an

innermost layer containing cells, which provide a soft surface and facilitate sliding, (2) a middle layer consisting of densely packed collagen and vessels, and (3) an outermost layer, made of fibrous tissue and also rich in vascularity (Hunter et al. 1983; Muangsanit et al. 2018). The vascularized pseudosheath can usually be harvested around 7–21 days following conduit implantation and serve as a nerve graft on its own (Wolford and Stevao 2003; Zadegan et al. 2015b). It can also be filled with proregenerative substances or placed within a nerve conduit to facilitate nerve regeneration (Muangsanit et al. 2018). There are several reports of successful nerve reconstruction by means of a vascularized pseudosheath in rat models, covering distances up to 15 mm (Lundborg and Hansson 1980; Penna et al. 2011; Zadegan et al. 2015a; Yapici et al. 2017). Penna and colleagues found a markedly higher number of blood vessels within the newly formed pseudosheath when compared to an autologous nerve graft. Additionally, axonal cross-sectional area was significantly increased compared to the autologous nerve graft following successful regrowth of the reconstructed nerve (Penna et al. 2011). Yapici's work provided the finding that both electrophysiological and histological parameters were more favorable when using a vascularized pseudosheath instead of a nonvascularized conduit to reconstruct the rat sciatic nerve. As was recently summarized by Muangsanit, the primary advantage of vascularized biogenic conduits is the avoidance of harvesting a donor nerve and therefore prevention of donor site morbidity (Muangsanit et al. 2018). Due to their favorable structural properties, regeneration-supporting features and superior vascularization, nerve regeneration, can be markedly enhanced (Zadegan et al. 2015b; Muangsanit et al. 2018). On top of that, scar formation and adhesions, two factors known for their deleterious effects on peripheral nerve function (Lemke et al. 2017; Crosio et al. 2021), are also reduced by the vascularized pseudosheath (Yapici et al. 2017). Nevertheless, a two-stage procedure for nerve reconstruction by means of these conduits is required, prolonging the time gap until reinnervation of target organs, one of the most crucial factors regarding functional recovery following nerve injury (Sakuma et al. 2016; Ronchi et al. 2017; Muangsanit et al. 2018; Sarhane et al. 2021).

8.5 Enhancement of Vascularization by Surgical Angiogenesis

Besides implantation of peripheral nerve grafts in proximity to an AV loop, enhanced vascularization can also be achieved by transferring a blood vessel-containing adipofascial flap to the site of nerve grafting as was recently demonstrated by Saffari et al. (2020a). This approach, termed “surgical angiogenesis,” increased vascularity of transplanted allografts and also resulted in an alteration of immunotrophic factors both locally and in the blood stream. Use of the aforementioned adipofascial flap in combination with allograft repair of the sciatic nerve in rats revealed an increased vascular diameter and vascular volume in comparison both to autograft repair and allograft repair without use of an adipofascial flap (Saffari et al. 2020c, d). Use of surgical angiogenesis might therefore become a regularly used tool to enhance nerve

regeneration, especially in a scarred wound bed with limited or low capacity for centripetal vascularization of nerve grafts (Saffari et al. 2020b).

8.6 Bioengineered Vascularized Neural Tissues

To overcome the problem of long gap nerve repair, bioengineered neural tissues have emerged as a promising alternative to provide vascularization. Nevertheless, the number of studies reporting results of nerve reconstruction by means of engineered constructs with improved vascularization remains limited (Muangsanit et al. 2018).

Gao and colleagues combined Schwann cells and vascular endothelial cells of rabbit origin, which they injected inside a nerve guidance conduit. These constructs facilitated nerve regeneration in a 2 cm rabbit sciatic nerve injury model both in regard to histomorphometric and electrophysiological parameters (Gao et al. 2013). The proregenerative capabilities of endothelial cells were further confirmed in an experiment conducted by Gingras et al., in which enhanced neurite outgrowth in vitro was observed in collagen-chitosan sponges loaded with endothelial cells (Gingras et al. 2003) (see chapter ► “Collagen Biomaterials for Nerve Tissue Engineering”).

Several other groups have attempted to generate microvascular networks in vitro, using techniques like (stereo)lithography (Whitesides et al. 2001; Sarker et al. 2018), photolithography (Kaihara et al. 2000; Curley and Moore 2011; Khoshakhlagh and Moore 2015; Lowry Curley and Moore 2017), and micropatterning (Folch and Toner 2000; Muangsanit et al. 2018).

Formation of endothelial cells into tube-like structures in vitro can also be achieved in carrier substances such as hydrogels or collagen, either by co-culturing them with other cell types such as pericytes or fibroblasts or by stimulating them with angiogenic growth factor (Berthod et al. 2006, 2012). These vascularized scaffolds can then be further enhanced by means of Schwann cells or other proregenerative molecules and cells (Georgiou et al. 2013, 2015; Martens et al. 2014; O’Rourke et al. 2015, 2018; Sanen et al. 2017; Muangsanit et al. 2018, 2020; Coy et al. 2020). Muangsanit and colleagues used tethered hydrogels seeded with either (1) Schwann cells, (2) human umbilical vein endothelial cells (HUVECs), or (3) both in co-culture to bridge a 10 mm defect of the rat sciatic nerve. They not only found that this approach significantly promoted nerve regeneration, but also that the HUVEC-containing constructs were superior to those seeded with Schwann cells or both cell types in combination (Muangsanit et al. 2021). Therefore, aligned HUVECs might potentially emerge at the forefront of strategies to enhance peripheral nerve regeneration (Grasman and Kaplan 2017).

Three-dimensional bioprinting of nerves and vascularized tissues is another emerging concept in the field of tissue engineering (Hann et al. 2019; Soman and Vijayavenkataraman 2020; Zhang et al. 2021b). Vascularization of engineered tissues is a highly intricate procedure due to the complexity of combining vasculature-specific and tissue-specific cells. Three-dimensional bioprinting is a versatile method able to tackle these challenges. Until now, despite rapidly emerging

advances in the field of 3D-printing, these approaches have not yet tackled the frontier of experimental or clinical application (Selim et al. 2021). However, in an era of high-resolution imaging of peripheral nerves (Heimel et al. 2019; Yan et al. 2019) to delineate their internal fascicular topography and microvascular structure to serve as template for bioengineered constructs, this is only a matter of time (Selim et al. 2021). Future technical advances will potentially ensure the development of vascularized conduits individualized in terms of fascicular architecture and vascular structure, catapulting nerve repair by means of engineered neural tissues into a new era of highly personalized treatment options. By adding a fourth dimension (Saska et al. 2021), e.g., printed materials able to change their conformation in response to cellular or molecular stimuli, the vision of patient-specific nerve repair can be approached one critical step further (Bova et al. 2020).

Apart from providing vascularization by means of structural compounds, e.g., tubular formations of HUVECs, embedding of angiogenetic factors is an additional vascularization strategy in the armamentarium of tissue-engineers. While the original vasculature or prefabricated vascular constructs help to “plug” the peripheral nerve graft or conduit into the vascular system, angiogenetic factors promote local formation of blood vessels, thereby facilitating incorporation of the respective nerve conduit with the original vasculature (Muangsanit et al. 2018; Zhao et al. 2019; Mastrullo et al. 2020).

As briefly stated previously, VEGF is the most commonly used angiogenic factor to enhance peripheral nerve regeneration, due to its crucial role in vascularization and its various interactions with other cell types required for peripheral nerve regeneration (Muangsanit et al. 2018). It can either be administered directly, e.g., intravenously or via a local injection (Fang et al. 2020; Masgutov et al. 2021) or be embedded for local release from nerve conduits (Zhao et al. 2019). Several studies reported both an increase in blood vessel counts as well as axon number following VEGF release from nerve constructs (Sondell et al. 1999; Hobson et al. 2000; Hobson 2002; Mohammadi et al. 2013; Li et al. 2020; Xia and Lv 2018) or acellular nerve grafts (Bin et al. 2020). Wu et al. embedded VEGF-overexpressing Schwann cells in a spongy conduit and found that with such conduits, Schwann cells showed improved proliferation, migration, and differentiation ability. In addition, nerve regeneration was excellent both in regard to morphological and functional parameters (Wu et al. 2021).

However, various clinical trials with the intent to enhance vascularization and in turn neural regeneration by administration of VEGF produced ambiguous results, emphasizing the complex interplay of angiogenesis and the other aforementioned mechanisms involved in neural regeneration (Best and Mackinnon 1994; Pereira Lopes et al. 2011; Hillenbrand et al. 2015; D’Arpa et al. 2015; Saffari et al. 2020b). This is also in line with the findings of Lee’s group, which administered VEGF via an osmotic pump after autograft repair of a 10 mm defect of the sciatic nerve. While formation of new blood vessels was enhanced, this did not translate into better motor recovery (Lee et al. 2016), emphasizing the importance of functional recovery over histomorphometric outcome in studies of peripheral nerve repair and regeneration (de Medinaceli et al. 1982). Hillebrand and colleagues locally administered VEGF

following spinal root C5 and C6 crush in newborn rats. While they observed a significant improvement of motor-function as well as of histomorphological parameters, they concluded that these effects were not primarily related to the increased vascularization caused by VEGF administration, but likely a combination of angiogenic, neurotrophic, and neuroprotective effects (Hillenbrand et al. 2015).

Besides VEGF, other factors and molecules for induction of vascularization or its support can be administered, such as nerve growth factor (NGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF) (Carmeliet 2000; Nomi et al. 2002), or other novel, small angiogenic molecules (Wiegand et al. 2006; Das et al. 2019; Muangsant et al. 2018).

8.7 Delivery of Stem Cells to Improve Vascularization

Stem cells are frequently used as therapeutics in experimental studies of tissue engineering in general (Kwon et al. 2018) and of peripheral nerve repair in particular (Fairbairn et al. 2015; Yi et al. 2020; Zhang et al. 2021a). However, data regarding their targeted application for improving vascularization is sparse in relation to the overall number of published studies. Saffari and colleagues summarized the current body of knowledge regarding this specific application in a recent review, addressing the yet unexplored exact interaction of vascularity and stem cells in peripheral nerve regeneration. As pointed out by the aforementioned authors, besides the stem cells' myelination-promoting effects and their capability to differentiate into Schwann-like cells, distinct signaling pathways lead to secretion of several angiogenic factors (Rehman et al. 2004; Hong et al. 2010; Watt et al. 2013), promoting angiogenesis (Saffari et al. 2021).

Stem cell delivery to enhance nerve regeneration has been extensively studied in experimental rodent (Zhao et al. 2011; Fan et al. 2014a, b; Mathot et al. 2020a, b; Saffari et al. 2020d, 2021; Krug et al. 2019) and large animal models including dogs (Ding et al. 2010), goats (Muheremu et al. 2017), and monkeys (Hu et al. 2013; Saffari et al. 2021). However, as was pointed out by Saffari, the exact survivability of these cells in vivo has not been elucidated in detail with one report of traceable bioluminescence for up to 29 days after seeding them on nerve allografts (Rbia et al. 2019). Additionally, there are only few studies investigating the role of stem cells and vascularity in peripheral nerve regeneration. Petrova et al. analyzed the number of blood vessels following sciatic nerve crush injury and subsequent subperineurial delivery of mesenchymal stem cells. They found that the blood vessel count was about 1.5 times higher 3 weeks after injury in comparison to a damaged nerve without stem cell delivery (Petrova et al. 2018). Mathot and colleagues obtained adipose-derived mesenchymal stem cells (MSCs) from Lewis rats, seeded them on allografts harvested from Sprague Dawley rats and then used these constructs to bridge a 10 mm sciatic nerve defect in Lewis rats. The authors observed that the formation of new vessels in decellularized nerve allografts was promoted by stem cells, in particular by differentiated MSCs, when compared to autologous nerve grafts. Additionally, the vascularization pattern in allografts seeded with MSCs

consisted of a large network of microvessels, which were not aligned and with a centripetal pattern, while the vessels in autografts and normal control nerves were more longitudinally aligned and showed longitudinal inosculation patterns (Mathot et al. 2020a, b).

Although preclinical investigations of stem cell applications eventually facilitated clinical investigations, use of this approach in regard to peripheral nerve injuries is still sparse. While the potential of therapeutic stem cells has been investigated in regard to burn wound healing, hemifacial spasm, and diabetic peripheral neuropathy, their potential for peripheral nerve regeneration and especially their role in vascularization in this context remains to be elucidated (Saffari et al. 2021).

9 Conclusions

Vascularization and perfusion are critical parts of the finely orchestrated processes of nerve function and regeneration. Anatomy and physiology of the peripheral nerve vascular system is highly complex and despite reasonable expectations, increased neural blood flow is more common than ischemia following nerve injury. Although decades of research have helped to understand specific features of peripheral nerve vasculature, e.g., the extrinsic and intrinsic system, other components such as the lymphatic system, remain to be elucidated in detail. Nevertheless, although not yet fully understood, microcirculation is heavily involved in peripheral nerve pathology. Therefore, strategies to promote vascularization and improve microcirculation of peripheral nerves are an indispensable tool for successful nerve regeneration and repair, especially in large nerve gaps. Despite a rapidly evolving body of knowledge regarding the anatomy and architecture of the vasa nervorum, our understanding of the fine interactions of these with the other components of the nerve is still lacking. While the technical and bioengineering options to enhance vascularization of peripheral nerves are steadily advancing, we must make sure to also deepen our knowledge of the complex interactions and effects exerted by the vasa nervorum. Only then can we achieve a biological solution for nerve repair, which is equivalent to the original nerve.

References

- Adams WE (1942) The blood supply of nerves: I. Historical review. *J Anat* 76:323–341
- Adams WE (1943) The blood supply of nerves: II. The effects of exclusion of its regional sources of supply on the sciatic nerve of the rabbit. *J Anat* 77:243–250.3
- Akhavani MA, Sivakumar B, Paleolog EM, Kang N (2008) Angiogenesis and plastic surgery. *J Plast Reconstr Aesthet Surg* 61:1425–1437
- Aloe L, Rocco ML, Bianchi P, Manni L (2012) Nerve growth factor: from the early discoveries to the potential clinical use. *J Transl Med* 10:239
- Appenzeller O, Dhital KK, Cowen T, Burnstock G (1984) The nerves to blood vessels supplying blood to nerves: the innervation of vasa nervorum. *Brain Res* 304:383–386

- Arányi Z, Csillik A, Dévay K, Rosero M (2018) Ultrasonographic demonstration of intraneural neovascularization after penetrating nerve injury. *Muscle Nerve* 57:994–999
- Bassiliou Habre S, Bond G, Jing XL, Kostopoulos E, Wallace RD, Konofaos P (2018) The surgical management of nerve gaps: present and future. *Ann Plast Surg* 80:252–261
- Bates D, Taylor GI, Minichiello J, Farlie P, Cichowitz A, Watson N, Klagsbrun M, Mamluk R, Newgreen DF (2003) Neurovascular congruence results from a shared patterning mechanism that utilizes semaphorin3A and neuropilin-1. *Dev Biol* 255:77–98
- Bäumer P, Reimann M, Decker C, Radbruch A, Bendszus M, Heiland S, Pham M (2014) Peripheral nerve perfusion by dynamic contrast-enhanced magnetic resonance imaging: demonstration of feasibility. *Investig Radiol* 49:518–523
- Bell MA, Weddell AG (1984a) A descriptive study of the blood vessels of the sciatic nerve in the rat, man and other mammals. *Brain* 107(Pt 3):871–898
- Bell MA, Weddell AG (1984b) A morphometric study of intrafascicular vessels of mammalian sciatic nerve. *Muscle Nerve* 7:524–534
- Bennett GJ, Xie YK (1988) A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 33:87–107
- Bertelli JA, Ghizoni MF (2007) Transfer of the accessory nerve to the suprascapular nerve in brachial plexus reconstruction. *J Hand Surg Am* 32:989–998
- Bertelli JA, Taleb M, Mira JC, Calixto JB (1996) Muscle fiber type reorganization and behavioral functional recovery of rat median nerve repair with vascularized or conventional nerve grafts. *Restor Neurol Neurosci* 10:5–12
- Berthod F, Germain L, Tremblay N, Auger FA (2006) Extracellular matrix deposition by fibroblasts is necessary to promote capillary-like tube formation in vitro. *J Cell Physiol* 207:491–498
- Berthod F, Symes J, Tremblay N, Medin JA, Auger FA (2012) Spontaneous fibroblast-derived pericyte recruitment in a human tissue-engineered angiogenesis model in vitro. *J Cell Physiol* 227:2130–2137
- Best TJ, Mackinnon SE (1994) Peripheral nerve revascularization: a current literature review. *J Reconstr Microsurg* 10:193–204
- Best TJ, Mackinnon SE, Evans PJ, Hunter D, Midha R (1999a) Peripheral nerve revascularization: histomorphometric study of small- and large-caliber grafts. *J Reconstr Microsurg* 15:183–190
- Best TJ, Mackinnon SE, Midha R, Hunter DA, Evans PJ (1999b) Revascularization of peripheral nerve autografts and allografts. *Plast Reconstr Surg* 104:152–160
- Bianchi R, Russo E, Bachmann SB, Proulx ST, Sesartic M, Smaadahl N, Watson SP, Buckley CD, Halin C, Detmar M (2017) Postnatal deletion of podoplanin in lymphatic endothelium results in blood filling of the lymphatic system and impairs dendritic cell migration to lymph nodes. *Arterioscler Thromb Vasc Biol* 37:108–117
- Bin Z, Zhihu Z, Jianxiong M, Xinlong M (2020) Repairing peripheral nerve defects with revascularized tissue-engineered nerve based on a vascular endothelial growth factor-heparin sustained release system. *J Tissue Eng Regen Med* 14:819–828
- Boissaud-Cooke M, Pidgeon TE, Tunstall R (2015) The microcirculation of peripheral nerves: the vasa nervorum. *Nerves and Nerve Injuries* 1:507–523
- Borire AA, Visser LH, Padua L, Colebatch JG, Huynh W, Simon NG, Kiernan MC, Krishnan AV (2017) Utility of maximum perfusion intensity as an ultrasonographic marker of intraneural blood flow. *Muscle Nerve* 55:77–83
- Bosetti F, Galis ZS, Bynoe MS, Charette M, Cipolla MJ, Del Zoppo GJ, Gould D, Hatsukami TS, Jones TLZ, Koenig JI, Luty GA, Maric-Bilkan C, Stevens T, Tolunay HE, Koroshetz W, Agalliu D, D'Amato R, Lo EH, Aird W, Antonetti DA, Boehm M, Brooks CE, Faber JE, Caron KM, Chilian W, Daemen MJ, Davis TP, Ergul A, Gomez AR, Peirce-Cottler S, Grayson P, Grumbach I, Suarez Y, Stachenfeld N, Humphrey J, Grutzendler J, Gutterman D, Ramadan I, McGavern D, Hallenbeck J, Herman I, Iadecola C, Ubogu EE, Inscho EW, Kleinfeld D, Lopez JA, Macknik S, Malik A, Meininger GA, Miller VM, Nedergaard M, Nelson MT, Rosenberg GA, Schiffrin EL, Searson P, Stan RV, Vexler ZS, Weyand CM,

- Zlokovic BV (2016) “Small blood vessels: big health problems?”: scientific recommendations of the National Institutes of Health Workshop. *J Am Heart Assoc* 5:1–12
- Bouvrée K, Brunet I, Del Toro R, Gordon E, Prahst C, Cristofaro B, Mathivet T, Xu Y, Soueid J, Fortuna V, Miura N, Aigrot MS, Maden CH, Ruhrberg C, Thomas JL, Eichmann A (2012) Semaphorin3A, neuropilin-1, and plexinA1 are required for lymphatic valve formation. *Circ Res* 111:437–445
- Bova L, Billi F, Cimetia E (2020) Mini-review: advances in 3D bioprinting of vascularized constructs. *Biol Direct* 15:22
- Breidenbach W, Terzis JK (1984) The anatomy of free vascularized nerve grafts. *Clin Plast Surg* 11: 65–71
- Breidenbach WC, Terzis JK (1986) The blood supply of vascularized nerve grafts. *J Reconstr Microsurg* 3:43–58
- Breidenbach WC, Terzis JK (1987) Vascularized nerve grafts: an experimental and clinical review. *Ann Plast Surg* 18:137–146
- Brunet I, Gordon E, Han J, Cristofaro B, Broqueres-You D, Liu C, Bouvrée K, Zhang J, Del Toro R, Mathivet T, Larrivée B, Jagu J, Pibouin-Fragner L, Pardanaud L, Machado MJ, Kennedy TE, Zhuang Z, Simons M, Levy BI, Tessier-Lavigne M, Grenz A, Eltzhig H, Eichmann A (2014) Netrin-1 controls sympathetic arterial innervation. *J Clin Invest* 124:3230–3240
- Caillaud M, Richard L, Vallat JM, Desmouliere A, Billet F (2019) Peripheral nerve regeneration and intraneural revascularization. *Neural Regen Res* 14:24–33
- Campbell WW (2008) Evaluation and management of peripheral nerve injury. *Clin Neurophysiol* 119:1951–1965
- Carmeliet P (2000) Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 6:389–395
- Castets M, Mehlen P (2010) Netrin-1 role in angiogenesis: to be or not to be a pro-angiogenic factor? *Cell Cycle* 9:1466–1471
- Cattin AL, Burden JJ, Van Emmenis L, Mackenzie FE, Hoving JJ, Garcia Calavia N, Guo Y, McLaughlin M, Rosenberg LH, Quereda V, Jamecna D, Napoli I, Parrinello S, Enver T, Ruhrberg C, Lloyd AC (2015) Macrophage-induced blood vessels guide Schwann cell-mediated regeneration of peripheral nerves. *Cell* 162:1127–1139
- Cavadas PC, Vera-Sempere FJ (1994) Prefabrication of a vascularized nerve graft by vessel implantation: preliminary report of an experimental model. *Microsurgery* 15:877–881
- Chalfoun C, Scholz T, Cole MD, Steward E, Vanderkam V, Evans GR (2003) Primary nerve grafting: a study of revascularization. *Microsurgery* 23:60–65
- Chang K, Ido Y, LeJeune W, Williamson JR, Tilton RG (1997) Increased sciatic nerve blood flow in diabetic rats: assessment by “molecular” vs. particulate microspheres. *Am J Physiol* 273: E164–E173
- Chauvet S, Mann F (2013) The declaration of independence of the neurovascular intimacy. *Neuron* 80:262–265
- Chen SH, Huang TC, Wang JY, Wu CC, Hsueh YY (2020) Controllable forces for reproducible chronic constriction injury mimicking compressive neuropathy in rat sciatic nerve. *J Neurosci Methods* 335:108615
- Chhabra A (2015) Advanced imaging of peripheral nerves. *Semin Musculoskelet Radiol* 19:77–78
- Coppey LJ, Gellett JS, Davidson EP, Dunlap JA, Lund DD, Yorek MA (2001) Effect of antioxidant treatment of streptozotocin-induced diabetic rats on endoneurial blood flow, motor nerve conduction velocity, and vascular reactivity of epineurial arterioles of the sciatic nerve. *Diabetes* 50:1927–1937
- Coppey LJ, Davidson EP, Rinehart TW, Gellett JS, Oltman CL, Lund DD, Yorek MA (2006) ACE inhibitor or angiotensin II receptor antagonist attenuates diabetic neuropathy in streptozotocin-induced diabetic rats. *Diabetes* 55:341–348
- Coy R, Al-Badri G, Kayal C, O'Rourke C, Kingham PJ, Phillips JB, Shipley RJ (2020) Combining in silico and in vitro models to inform cell seeding strategies in tissue engineering. *J R Soc Interface* 17:20190801

- Crosio A, Ronchi G, Fornasari BE, Odella S, Raimondo S, Tos P (2021) Experimental methods to simulate and evaluate postsurgical peripheral nerve scarring. *J Clin Med* 10:1613
- Curley JL, Moore MJ (2011) Facile micropatterning of dual hydrogel systems for 3D models of neurite outgrowth. *J Biomed Mater Res A* 99:532–543
- D'Arpa S, Claes KEY, Stillaert F, Colebunders B, Monstrey S, Blondeel P (2015) Vascularized nerve “grafts”: just a graft or a worthwhile procedure? *Plast Aesthet Res* 2:183–194
- Daly PJ, Wood MB (1985) Endoneural and epineural blood flow evaluation with free vascularized and conventional nerve grafts in the canine. *J Reconstr Microsurg* 2:45–49
- Das A, Merrill P, Wilson J, Turner T, Paige M, Capitosti S, Brown M, Freshcorn B, Sok MCP, Song H, Botchwey EA (2019) Evaluating angiogenic potential of small molecules using genetic network approaches. *Regen Eng Transl Med* 5:30–41
- de Medinaceli L, Freed WJ, Wyatt RJ (1982) An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. *Exp Neurol* 77:634–643
- del Pinal F, Garcia-Bernal FJ, Regalado J, Studer A, Cagigal L, Ayala H (2007) The tibial second toe vascularized neurocutaneous free flap for major digital nerve defects. *J Hand Surg Am* 32:209–217
- Dhital KK, Appenzeller O (1998) Innervation of the vasa nervorum. In: Burnstock G, Griffith SG (eds) *Nonadrenergic innervation of blood vessels*. CRC Press, Boca Raton
- Ding F, Wu J, Yang Y, Hu W, Zhu Q, Tang X, Liu J, Gu X (2010) Use of tissue-engineered nerve grafts consisting of a chitosan/poly(lactic-co-glycolic acid)-based scaffold included with bone marrow mesenchymal cells for bridging 50-mm dog sciatic nerve gaps. *Tissue Eng Part A* 16:3779–3790
- Doerffler J (1768) *De Vasis Nervorum*. Thesis, University of Erlangen
- Doi K, Kuwata N, Sakai K, Tamaru K, Kawai S (1987) A reliable technique of free vascularized sural nerve grafting and preliminary results of clinical applications. *J Hand Surg Am* 12:677–684
- Doi K, Tamaru K, Sakai K, Kuwata N, Kurafuji Y, Kawai S (1992) A comparison of vascularized and conventional sural nerve grafts. *J Hand Surg Am* 17:670–676
- Donzelli R, Capone C, Sgulo FG, Mariniello G, Maiuri F (2016) Vascularized nerve grafts: an experimental study. *Neurol Res* 38:669–677
- Durward A (1948) The blood supply of nerves. *Postgrad Med J* 24:11–14
- Dyck PJ (1989) Hypoxic neuropathy: does hypoxia play a role in diabetic neuropathy? The 1988 Robert Wartenberg lecture. *Neurology* 39:111–118
- Dyck PJ, Giannini C (1996) Pathologic alterations in the diabetic neuropathies of humans: a review. *J Neuropathol Exp Neurol* 55:1181–1193
- Dyck PJ, Hansen S, Karnes J, O'Brien P, Yasuda H, Windebank A, Zimmerman B (1985) Capillary number and percentage closed in human diabetic sural nerve. *Proc Natl Acad Sci U S A* 82:2513–2517
- Dyck PJ, Karnes JL, O'Brien P, Okazaki H, Lais A, Engelstad J (1986) The spatial distribution of fiber loss in diabetic polyneuropathy suggests ischemia. *Ann Neurol* 19:440–449
- Dyck PJ, Lais AC, Giannini C, Engelstad JK (1990) Structural alterations of nerve during cuff compression. *Proc Natl Acad Sci U S A* 87:9828–9832
- Eichmann A, Brunet I (2014) Arterial innervation in development and disease. *Sci Transl Med* 6:252ps9
- El-Barrany WG, Marei AG, Vallée B (1999) Anatomic basis of vascularised nerve grafts: the blood supply of peripheral nerves. *Surg Radiol Anat* 21:95–102
- Evans KD, Roll SC, Volz KR, Freimer M (2012) Relationship between intraneural vascular flow measured with sonography and carpal tunnel syndrome diagnosis based on electrodiagnostic testing. *J Ultrasound Med* 31:729–736
- Fairbairn NG, Meppelink AM, Ng-Glazier J, Randolph MA, Winograd JM (2015) Augmenting peripheral nerve regeneration using stem cells: a review of current opinion. *World J Stem Cells* 7:11–26
- Falco NA, Pribaz JJ, Eriksson E (1992) Vascularization of skin following implantation of an arteriovenous pedicle: implications in flap prefabrication. *Microsurgery* 13:249–254

- Fan L, Yu Z, Li J, Dang X, Wang K (2014a) Immunoregulation effects of bone marrow-derived mesenchymal stem cells in xenogeneic acellular nerve grafts transplant. *Cell Mol Neurobiol* 34: 999–1010
- Fan L, Yu Z, Li J, Dang X, Wang K (2014b) Schwann-like cells seeded in acellular nerve grafts improve nerve regeneration. *BMC Musculoskelet Disord* 15:165
- Fan AP, Jahanian H, Holdsworth SJ, Zaharchuk G (2016) Comparison of cerebral blood flow measurement with [¹⁵O]-water positron emission tomography and arterial spin labeling magnetic resonance imaging: a systematic review. *J Cereb Blood Flow Metab* 36:842–861
- Fang Z, Ge X, Chen X, Xu Y, Yuan WE, Ouyang Y (2020) Enhancement of sciatic nerve regeneration with dual delivery of vascular endothelial growth factor and nerve growth factor genes. *J Nanobiotechnol* 18:46
- Farzan M, Mortazavi SMJ, Asadollahi S (1970) Cubital tunnel syndrome: review of 14 anterior subcutaneous transpositions of the vascularized ulnar nerve. *Acta Med Iran* 43:84
- Ferretti A, Boschi E, Stefani A, Spiga S, Romanelli M, Lemmi M, Giovannetti A, Longoni B, Mosca F (2003) Angiogenesis and nerve regeneration in a model of human skin equivalent transplant. *Life Sci* 73:1985–1994
- Fink DM, Connor AL, Kelley PM, Steele MM, Hollingsworth MA, Tempero RM (2014) Nerve growth factor regulates neurolymphatic remodeling during corneal inflammation and resolution. *PLoS One* 9:e112737
- Firrell J (2019) Peripheral nerve microcirculation. In: Clinically applied microcirculation research. Routledge, London
- Folch A, Toner M (2000) Microengineering of cellular interactions. *Annu Rev Biomed Eng* 2:227–256
- Foo A, Martin-Playa P, Sebastin Muttath SJ (2019) Arterialized posterior interosseous nerve graft for digital neuroma. *Tech Hand Up Extrem Surg* 23:152–154
- Frueh FS, Gousopoulos E, Power DM, Ampofo E, Giovanoli P, Calcagni M, Laschke MW (2020) A potential role of lymphangiogenesis for peripheral nerve injury and regeneration. *Med Hypotheses* 135:109470
- Gao H, You Y, Zhang G, Zhao F, Sha Z, Shen Y (2013) The use of fiber-reinforced scaffolds cocultured with Schwann cells and vascular endothelial cells to repair rabbit sciatic nerve defect with vascularization. *Biomed Res Int* 2013:362918
- Garozzo D, Ferraresi S, Buffatti P (2004) Surgical treatment of common peroneal nerve injuries: indications and results. A series of 62 cases. *J Neurosurg Sci* 48:105–112. Discussion 112
- Georgiou M, Bunting SC, Davies HA, Loughlin AJ, Golding JP, Phillips JB (2013) Engineered neural tissue for peripheral nerve repair. *Biomaterials* 34:7335–7343
- Georgiou M, Golding JP, Loughlin AJ, Kingham PJ, Phillips JB (2015) Engineered neural tissue with aligned, differentiated adipose-derived stem cells promotes peripheral nerve regeneration across a critical sized defect in rat sciatic nerve. *Biomaterials* 37:242–251
- Gerard RW (1927) Studies on nerve metabolism: I. The influence of oxygen lack on heat production and action current. *J Physiol* 63:280–298
- Gerard RW (1930) The oxygen consumption of nerve during activity. *Science* 72:195–196
- Gerard RW, Hill AV, Zotterman Y (1927) The effect of frequency of stimulation on the heat production of nerve. *J Physiol* 63:130–143
- Giannini C, Dyck PJ (1995) Basement membrane reduplication and pericyte degeneration precede development of diabetic polyneuropathy and are associated with its severity. *Ann Neurol* 37: 498–504
- Gingras M, Bergeron J, Dery J, Durham HD, Berthod F (2003) In vitro development of a tissue-engineered model of peripheral nerve regeneration to study neurite growth. *FASEB J* 17: 2124–2126
- Glebova NO, Ginty DD (2005) Growth and survival signals controlling sympathetic nervous system development. *Annu Rev Neurosci* 28:191–222
- Goedee HS, Brekelmans GJ, van Asseldonk JT, Beekman R, Mess WH, Visser LH (2013) High resolution sonography in the evaluation of the peripheral nervous system in polyneuropathy – a review of the literature. *Eur J Neurol* 20:1342–1351

- Gordon T, Tyreman N, Raji MA (2011) The basis for diminished functional recovery after delayed peripheral nerve repair. *J Neurosci* 31:5325–5334
- Graf P, Hawe W, Biemer E (1986) Vascular supply of the ulnar nerve following neurolysis in the area of the elbow. *Handchir Mikrochir Plast Chir* 18:204–206
- Grasman JM, Kaplan DL (2017) Human endothelial cells secrete neurotropic factors to direct axonal growth of peripheral nerves. *Sci Rep* 7:4092
- Gu X, Ding F, Yang Y, Liu J (2011) Construction of tissue engineered nerve grafts and their application in peripheral nerve regeneration. *Prog Neurobiol* 93:204–230
- Gugala Z, Olmsted-Davis EA, Xiong Y, Davis EL, Davis AR (2018) Trauma-induced heterotopic ossification regulates the blood-nerve barrier. *Front Neurol* 9:408
- Hann SY, Cui H, Esworthy T, Miao S, Zhou X, Lee SJ, Fisher JP, Zhang LG (2019) Recent advances in 3D printing: vascular network for tissue and organ regeneration. *Transl Res* 211:46–63
- Hattori Y, Doi K, Ikeda K, Pagsaligan JM (2005) Vascularized ulnar nerve graft for reconstruction of a large defect of the median or radial nerves after severe trauma of the upper extremity. *J Hand Surg Am* 30:986–989
- Heimel P, Swiadek NV, Slezak P, Kerbl M, Schneider C, Nürnberger S, Redl H, Teuschl AH, Hercher D (2019) Iodine-enhanced micro-CT imaging of soft tissue on the example of peripheral nerve regeneration. *Contrast Media Mol Imaging* 2019:7483745
- Heinzel JC, Gloeckel M, Gruber A, Heher P, Hercher D (2020) Fibrin in nerve tissue engineering. In: Phillips J, Hercher D, Hausner T (eds) *Peripheral nerve tissue engineering and regeneration*. Springer, Cham
- Helton ES, Palladino S, Ubogu EE (2017) A novel method for measuring hydraulic conductivity at the human blood-nerve barrier in vitro. *Microvasc Res* 109:1–6
- Henn D, Abu-Halima M, Kahraman M, Falkner F, Fischer KS, Barrera JA, Chen K, Gurtner GC, Keller A, Kneser U, Meese E, Schmidt VJ (2020a) A multivariable miRNA signature delineates the systemic hemodynamic impact of arteriovenous shunt placement in a pilot study. *Sci Rep* 10:21809
- Henn D, Chen K, Fischer K, Rauh A, Barrera JA, Kim YJ, Martin RA, Hannig M, Niedoba P, Reddy SK, Mao HQ, Kneser U, Gurtner GC, Sacks JM, Schmidt VJ (2020b) Tissue engineering of axially vascularized soft-tissue flaps with a poly(ϵ -caprolactone) nanofiber-hydrogel composite. *Adv Wound Care* 9:365–377
- Hess K, Eames RA, Darveniza P, Gilliatt RW (1979) Acute ischaemic neuropathy in the rabbit. *J Neurol Sci* 44:19–43
- Hillenbrand M, Holzbach T, Matiaszek K, Schlegel J, Giunta RE (2015) Vascular endothelial growth factor gene therapy improves nerve regeneration in a model of obstetric brachial plexus palsy. *Neurol Res* 37:197–203
- Hobson MI (2002) Increased vascularisation enhances axonal regeneration within an acellular nerve conduit. *Ann R Coll Surg Engl* 84:47–53
- Hobson MI, Brown R, Green CJ, Terenghi G (1997) Inter-relationships between angiogenesis and nerve regeneration: a histochemical study. *Br J Plast Surg* 50:125–131
- Hobson MI, Green CJ, Terenghi G (2000) VEGF enhances intraneural angiogenesis and improves nerve regeneration after axotomy. *J Anat* 197(Pt 4):591–605
- Höke A, Sun HS, Gordon T, Zochodne DW (2001) Do denervated peripheral nerve trunks become ischemic? The impact of chronic denervation on vasa nervorum. *Exp Neurol* 172:398–406
- Holzgrefe RE, Wagner ER, Singer AD, Daly CA (2019) Imaging of the peripheral nerve: concepts and future direction of magnetic resonance neurography and ultrasound. *J Hand Surg Am* 44:1066–1079
- Hong SJ, Traktuev DO, March KL (2010) Therapeutic potential of adipose-derived stem cells in vascular growth and tissue repair. *Curr Opin Organ Transplant* 15:86–91
- Horch RE, Beier JP, Kneser U, Arkudas A (2014) Successful human long-term application of in situ bone tissue engineering. *J Cell Mol Med* 18:1478–1485
- Hromada J (1963) On the nerve supply of the connective tissue of some peripheral nervous system components. *Acta Anat* 55:343–351

- Hu N, Wu H, Xue C, Gong Y, Wu J, Xiao Z, Yang Y, Ding F, Gu X (2013) Long-term outcome of the repair of 50 mm long median nerve defects in rhesus monkeys with marrow mesenchymal stem cells-containing, chitosan-based tissue engineered nerve grafts. *Biomaterials* 34:100–111
- Hunter JM, Jaeger SH, Matsui T, Miyaji N (1983) The pseudosynovial sheath – its characteristics in a primate model. *J Hand Surg Am* 8:461–470
- Hur J, Jang JH, Oh IY, Choi JI, Yun JY, Kim J, Choi YE, Ko SB, Kang JA, Kang J, Lee SE, Lee H, Park YB, Kim HS (2014) Human podoplanin-positive monocytes and platelets enhance lymphangiogenesis through the activation of the podoplanin/CLEC-2 axis. *Mol Ther* 22:1518–1529
- Hyrtl J (1846) *Lehrbuch der Anatomie des Menschen*. Prag, Verlag von Friedrich Ehrlich, p 816
- Iijima Y, Ajiki T, Murayama A, Takeshita K (2016) Effect of artificial nerve conduit vascularization on peripheral nerve in a necrotic bed. *Plast Reconstr Surg Glob Open* 4:e665
- Isaacs J, Browne T (2014) Overcoming short gaps in peripheral nerve repair: conduits and human acellular nerve allograft. *Hand* 9:131–137
- Jain RK, Au P, Tam J, Duda DG, Fukumura D (2005) Engineering vascularized tissue. *Nat Biotechnol* 23:821–823
- James JM, Mukoyama YS (2011) Neuronal action on the developing blood vessel pattern. *Semin Cell Dev Biol* 22:1019–1027
- Jessen KR, Mirsky R (2016) The repair Schwann cell and its function in regenerating nerves. *J Physiol* 594:3521–3531
- Joy V, Therimadasamy AK, Chan YC, Wilder-Smith EP (2011) Combined Doppler and B-mode sonography in carpal tunnel syndrome. *J Neurol Sci* 308:16–20
- Juriscic G, Maby-El Hajjami H, Karaman S, Ochsenbein AM, Alitalo A, Siddiqui SS, Ochoa Pereira C, Petrova TV, Detmar M (2012) An unexpected role of semaphorin3a–neuropilin-1 signaling in lymphatic vessel maturation and valve formation. *Circ Res* 111:426–436
- Kadiyala RK, Ramirez A, Taylor AE, Saltzman CL, Cassell MD (2005) The blood supply of the common peroneal nerve in the popliteal fossa. *J Bone Joint Surg Br* 87:337–342
- Kaihara S, Borenstein J, Koka R, Lalan S, Ochoa ER, Ravens M, Pien H, Cunningham B, Vacanti JP (2000) Silicon micromachining to tissue engineer branched vascular channels for liver fabrication. *Tissue Eng* 6:105–117
- Kaizawa Y, Kakinoki R, Ikeguchi R, Ohta S, Noguchi T, Oda H, Matsuda S (2016) Bridging a 30 mm defect in the canine ulnar nerve using vessel-containing conduits with implantation of bone marrow stromal cells. *Microsurgery* 36:316–324
- Kaizawa Y, Kakinoki R, Ikeguchi R, Ohta S, Noguchi T, Takeuchi H, Oda H, Yurie H, Matsuda S (2017) A nerve conduit containing a vascular bundle and implanted with bone marrow stromal cells and decellularized allogenic nerve matrix. *Cell Transplant* 26:215–228
- Kakinoki R, Nishijima N, Ueba Y, Oka M, Yamamuro T, Nakamura T (1997) Nerve regeneration over a 25 mm gap in rat sciatic nerves using tubes containing blood vessels: the possibility of clinical application. *Int Orthop* 21:332–336
- Kallio PK, Vastamaki M (1993) An analysis of the results of late reconstruction of 132 median nerves. *J Hand Surg Br* 18:97–105
- Kanaya F, Firrell J, Tsai TM, Breidenbach WC (1992) Functional results of vascularized versus nonvascularized nerve grafting. *Plast Reconstr Surg* 89:924–930
- Kaplan HM, Mishra P, Kohn J (2015) The overwhelming use of rat models in nerve regeneration research may compromise designs of nerve guidance conduits for humans. *J Mater Sci Mater Med* 26:226
- Khoshakhlagh P, Moore MJ (2015) Photoreactive interpenetrating network of hyaluronic acid and Puramatrix as a selectively tunable scaffold for neurite growth. *Acta Biomater* 16:23–34
- Kihara M, Low PA (1990) Regulation of rat nerve blood flow: role of epineurial alpha-receptors. *J Physiol* 422:145–152
- Kihara M, Low PA (1995) Vasoreactivity to prostaglandins of rat peripheral nerve. *J Physiol* 484:463–467

- Kihara M, Zollman PJ, Schmelzer JD, Low PA (1993) The influence of dose of microspheres on nerve blood flow, electrophysiology, and fiber degeneration of rat peripheral nerve. *Muscle Nerve* 16:1383–1389
- Kihara M, Nakasaka Y, Mitsui Y, Takahashi M, Schmelzer JD (2000) Aging differentially modifies sensitivity of nerve blood flow to vasocontractile agents (endothelin-1, noradrenaline and angiotensin II) in sciatic nerve. *Mech Ageing Dev* 114:5–14
- Kobayashi M, Zochodne DW (2018) Diabetic neuropathy and the sensory neuron: new aspects of pathogenesis and their treatment implications. *J Diabetes Investig* 9:1239–1254
- Koistinaho J, Wadhvani KC, Balbo A, Rapoport SI (1991) Regeneration of perivascular adrenergic innervation in rat tibial nerve after nerve crush. *Acta Neuropathol* 81:486–490
- Kolte D, McClung JA, Aronow WS (2016) Chapter 6. Vasculogenesis and angiogenesis. In: Aronow WS, McClung JA (eds) *Translational research in coronary artery disease*. Academic Press, Boston
- Kornfeld T, Vogt PM, Radtke C (2019) Nerve grafting for peripheral nerve injuries with extended defect sizes. *Wien Med Wochenschr* 169:240–251
- Korthals JK, Gieron MA, Dyck PJ (1988) Intima of epineurial arterioles is increased in diabetic polyneuropathy. *Neurology* 38:1582–1586
- Kosaka M (1990) Enhancement of rat peripheral nerve regeneration through artery-including silicone tubing. *Exp Neurol* 107:69–77
- Koshima I, Harii K (1985) Experimental study of vascularized nerve grafts: morphometric study of axonal regeneration of nerves transplanted into silicone tubes. *Ann Plast Surg* 14:235–243
- Krug C, Beer A, Hartmann B, Prein C, Clause-Schaumann H, Holzbach T, Aszodi A, Giunta RE, Saller MM, Volkmer E (2019) Fibrin glue displays promising in vitro characteristics as a potential carrier of adipose progenitor cells for tissue regeneration. *J Tissue Eng Regen Med* 13:359–368
- Kuhlmann T, Bitsch A, Stadelmann C, Siebert H, Bruck W (2001) Macrophages are eliminated from the injured peripheral nerve via local apoptosis and circulation to regional lymph nodes and the spleen. *J Neurosci* 21:3401–3408
- Kutlar N, Bayrak AO, Bayrak İK, Canbaz S, Türker H (2017) Diagnosing carpal tunnel syndrome with Doppler ultrasonography: a comparison of ultrasonographic measurements and electrophysiological severity. *Neurol Res* 39:126–132
- Kwon SG, Kwon YW, Lee TW, Park GT, Kim JH (2018) Recent advances in stem cell therapeutics and tissue engineering strategies. *Biomater Res* 22:36
- Kwon JG, Choi YJ, Kim SC, Hong JP, Jeong WS, Oh TS (2019) A technique for safe deep facial tissue dissection: Indocyanine green-assisted intraoperative real-time visualization of the vasa nervorum of facial nerve with a near-infrared camera. *J Cranio-Maxillofac Surg* 47:1819–1826
- Lagerlund TD, Low PA (1987) A mathematical simulation of oxygen delivery in rat peripheral nerve. *Microvasc Res* 34:211–222
- Lagerlund TD, Low PA (1993) Mathematical modeling of time-dependent oxygen transport in rat peripheral nerve. *Comput Biol Med* 23:29–47
- Lang J (1962) On connective tissue and blood vessels of the nerves. *Z Anat Entwicklungsgesch* 123:61–79
- Lang J (1964) Über die Gefäße, die Faszikel und das Bindegewebe der Nerven während des Wachstums. *Z Zellforsch Mikrosk Anat* 63:226–246
- Lanzetta M, Herbert TJ, Conolly WB (1994) Silicone synovitis. A perspective. *J Hand Surg Br* 19:479–484
- Lee JY, Giusti G, Friedrich PF, Bishop AT, Shin AY (2016) Effect of vascular endothelial growth factor administration on nerve regeneration after autologous nerve grafting. *J Reconstr Microsurg* 32:183–188
- Lemke A, Penzenstadler C, Ferguson J, Lidinsky D, Hopf R, Bradl M, Redl H, Wolbank S, Hausner T (2017) A novel experimental rat model of peripheral nerve scarring that reliably mimics post-surgical complications and recurring adhesions. *Dis Model Mech* 10:1015–1025

- Levy D, Zochodne DW (1998) Local nitric oxide synthase activity in a model of neuropathic pain. *Eur J Neurosci* 10:1846–1855
- Li W, Kohara H, Uchida Y, James JM, Soneji K, Cronshaw DG, Zou YR, Nagasawa T, Mukouyama YS (2013) Peripheral nerve-derived CXCL12 and VEGF-A regulate the patterning of arterial vessel branching in developing limb skin. *Dev Cell* 24:359–371
- Li R, Xu J, Rao Z, Deng R, Xu Y, Qiu S, Long H, Zhu Q, Liu X, Bai Y, Quan D (2020) Facilitate angiogenesis and neurogenesis by growth factors integrated decellularized matrix hydrogel. *Tissue Eng Part A* 27(11–12):771–787
- Lim TK, Shi XQ, Martin HC, Huang H, Luheshi G, Rivest S, Zhang J (2014) Blood-nerve barrier dysfunction contributes to the generation of neuropathic pain and allows targeting of injured nerves for pain relief. *Pain* 155:954–967
- Lind R, Wood MB (1986) Comparison of the pattern of early revascularization of conventional versus vascularized nerve grafts in the canine. *J Reconstr Microsurg* 2:229–234
- Lovati AB, D'Arrigo D, Odella S, Tos P, Geuna S, Raimondo S (2018) Nerve repair using decellularized nerve grafts in rat models a review of the literature. *Front Cell Neurosci* 12:427
- Low PA, Tuck RR (1984) Effects of changes of blood pressure, respiratory acidosis and hypoxia on blood flow in the sciatic nerve of the rat. *J Physiol* 347:513–524
- Low PA, Dyck PJ, Schmelzer JD (1980) Mammalian peripheral nerve sheath has unique responses to chronic elevations of endoneurial fluid pressure. *Exp Neurol* 70:300–306
- Low PA, Lagerlund TD, McManis PG (1989) Nerve blood flow and oxygen delivery in normal, diabetic, and ischemic neuropathy. *Int Rev Neurobiol* 31:355–438
- Lowry Curley J, Moore MJ (2017) 3D neural culture in dual hydrogel systems. *Methods Mol Biol* 1612:225–237
- Lundborg G (1968) Microvascular structure and function of peripheral nerves. Vital microscopic studies of the tibial nerve in the rabbit. *Adv Microcirc* 1:66–88
- Lundborg G (1970) Ischemic nerve injury. Experimental studies on intraneural microvascular pathophysiology and nerve function in a limb subjected to temporary circulatory arrest. *Scand J Plast Reconstr Surg Suppl* 6:3–113
- Lundborg G (1975) Structure and function of the intraneural microvessels as related to trauma, edema formation, and nerve function. *J Bone Joint Surg Am* 57:938–948
- Lundborg G (1988) Intraneural microcirculation. *Orthop Clin North Am* 19:1–12
- Lundborg G, Hansson HA (1980) Nerve regeneration through preformed pseudosynovial tubes. A preliminary report of a new experimental model for studying the regeneration and reorganization capacity of peripheral nerve tissue. *J Hand Surg Am* 5:35–38
- Lundborg G, Myers R, Powell H (1983) Nerve compression injury and increased endoneurial fluid pressure: a “miniature compartment syndrome”. *J Neurol Neurosurg Psychiatry* 46:1119–1124
- Luo YX, Wang TP (1992) A clinical application of artery-including silicone tubing to peripheral nerve defect. *J Tongji Med Univ* 12:247–249
- Mackinnon SE, Dellon AL (1986) Experimental study of chronic nerve compression. Clinical implications. *Hand Clin* 2:639–650
- Mackinnon SE, Dellon AL, Hudson AR, Hunter DA (1984) Chronic nerve compression – an experimental model in the rat. *Ann Plast Surg* 13:112–120
- Madison RD, Zomorodi A, Robinson GA (2000) Netrin-1 and peripheral nerve regeneration in the adult rat. *Exp Neurol* 161:563–570
- Mahan MA (2019) Nerve stretching: a history of tension. *J Neurosurg* 132:252–259
- Maki Y, Breidenbach WC, Firrell JC (1993) Evaluation of a local microsphere injection method for measurement of blood flow in the rabbit lower extremity. *J Orthop Res* 11:20–27
- Maki Y, Firrell JC, Breidenbach WC (1997) Blood flow in mobilized nerves: results in a rabbit sciatic nerve model. *Plast Reconstr Surg* 100(3):627–633
- Makita T, Sucov HM, Garipey CE, Yanagisawa M, Ginty DD (2008) Endothelins are vascular-derived axonal guidance cues for developing sympathetic neurons. *Nature* 452:759–763
- Malik RA (2014) Pathology of human diabetic neuropathy. *Handb Clin Neurol* 126:249–259

- Mani GV, Shurey C, Green CJ (1992) Is early vascularization of nerve grafts necessary? *J Hand Surg Br* 17:536–543
- Martens W, Sanen K, Georgiou M, Struys T, Bronckaers A, Ameloot M, Phillips J, Lambrichts I (2014) Human dental pulp stem cells can differentiate into Schwann cells and promote and guide neurite outgrowth in an aligned tissue-engineered collagen construct in vitro. *FASEB J* 28: 1634–1643
- Martin P, Lewis J (1989) Origins of the neurovascular bundle: interactions between developing nerves and blood vessels in embryonic chick skin. *Int J Dev Biol* 33:379–387
- Masgutov R, Zeinalova A, Bogov A, Masgutova G, Salafutdinov I, Garanina E, Syromiatnikova V, Idrisova K, Mullakhmetova A, Andreeva D, Mukhametova L, Kadyrov A, Pankov I, Rizvanov A (2021) Angiogenesis and nerve regeneration induced by local administration of plasmid pBud-coVEGF165-coFGF2 into the intact rat sciatic nerve. *Neural Regen Res* 16:1882–1889
- Mastrullo V, Cathery W, Velliou E, Madeddu P, Campagnolo P (2020) Angiogenesis in tissue engineering: as nature intended? *Front Bioeng Biotechnol* 8:188
- Mathot F, Rbia N, Bishop AT, Hovius SER, Shin AY (2020a) Adipose derived mesenchymal stem cells seeded onto a decellularized nerve allograft enhances angiogenesis in a rat sciatic nerve defect model. *Microsurgery* 40:585–592
- Mathot F, Rbia N, Thaler R, Bishop AT, Van Wijnen AJ, Shin AY (2020b) Gene expression profiles of differentiated and undifferentiated adipose derived mesenchymal stem cells dynamically seeded onto a processed nerve allograft. *Gene* 724:144151
- Matsumoto N (1983) Experimental study on compression neuropathy – determination of blood flow by a hydrogen washout technic. *Nihon Seikeigeka Gakkai Zasshi* 57:805–816
- McCullough CJ, Gagey O, Higginson DW, Sandin BM, Crow JC, Seville A (1984) Axon regeneration and vascularisation of nerve grafts. An experimental study. *J Hand Surg Br* 9:323–327
- McManis PG, Low PA (1988) Factors affecting the relative viability of centrifascicular and subperineurial axons in acute peripheral nerve ischemia. *Exp Neurol* 99:84–95
- McManis PG, Low PA, Yao JK (1986) Relationship between nerve blood flow and intercapillary distance in peripheral nerve edema. *Am J Physiol* 251:E92–E97
- Meng FW, Jing XN, Song GH, Jie LL, Shen FF (2020) Prox1 induces new lymphatic vessel formation and promotes nerve reconstruction in a mouse model of sciatic nerve crush injury. *J Anat* 237:933–940
- Merle M, Dautel G (1991) Vascularised nerve grafts. *J Hand Surg Br* 16:483–488
- Merolli A, Rocchi L, catalano F, Planell J, Engel E, Martinez E, Sbernadori MC, Marceddu S, Leali PT (2009) In vivo regeneration of rat sciatic nerve in a double-halved stitch-less guide: a pilot-study. *Microsurgery* 29:310–318
- Millesi H (1982) Peripheral nerve injuries. Nerve sutures and nerve grafting. *Scand J Plast Reconstr Surg Suppl* 19:25–37
- Millesi H (1992) *Chirurgie der peripheren Nerven*. Elsevier, München
- Millesi H (2000) Techniques for nerve grafting. *Hand Clin* 16:73–91, viii
- Millesi H (2007) Bridging defects: autologous nerve grafts. *Acta Neurochir Suppl* 100:37–38
- Millesi H, Zoch G, Rath T (1990) The gliding apparatus of peripheral nerve and its clinical significance. *Ann Chir Main Memb Super* 9:87–97
- Millesi H, Hausner T, Schmidhammer R, Trattnig S, Tschabitscher M (2007) Anatomical structures to provide passive motility of peripheral nerve trunks and fascicles. *Acta Neurochir Suppl* 100: 133–135
- Mizisin AP, Weerasuriya A (2011) Homeostatic regulation of the endoneurial microenvironment during development, aging and in response to trauma, disease and toxic insult. *Acta Neuropathol* 121:291–312
- Mohammadi R, Ahsan S, Masoumi M, Amini K (2013) Vascular endothelial growth factor promotes peripheral nerve regeneration after sciatic nerve transection in rat. *Chin J Traumatol* 16: 323–329
- Muangsanit P, Shipley RJ, Phillips JB (2018) Vascularization strategies for peripheral nerve tissue engineering. *Anat Rec* 301:1657–1667

- Muangsanit P, Day A, Dimiou S, Ataç AF, Kayal C, Park H, Nazhat SN, Phillips JB (2020) Rapidly formed stable and aligned dense collagen gels seeded with Schwann cells support peripheral nerve regeneration. *J Neural Eng* 17:046036
- Muangsanit P, Robertson V, Costa E, Phillips J (2021) Engineered aligned endothelial cell structures in tethered collagen hydrogels promote peripheral nerve regeneration. *Acta Biomater* 126:224–237
- Muheremu A, Chen L, Wang X, Wei Y, Gong K, Ao Q (2017) Chitosan nerve conduits seeded with autologous bone marrow mononuclear cells for 30 mm goat peroneal nerve defect. *Sci Rep* 7: 44002
- Mukouyama YS, Shin D, Britsch S, Taniguchi M, Anderson DJ (2002) Sensory nerves determine the pattern of arterial differentiation and blood vessel branching in the skin. *Cell* 109:693–705
- Mukouyama YS, Gerber HP, Ferrara N, Gu C, Anderson DJ (2005) Peripheral nerve-derived VEGF promotes arterial differentiation via neuropilin 1-mediated positive feedback. *Development* 132: 941–952
- Muratori L, Gnani S, Fregnan F, Mancardi A, Raimondo S, Perroteau I, Geuna S (2018) Evaluation of vascular endothelial growth factor (VEGF) and its family member expression after peripheral nerve regeneration and denervation. *Anat Rec* 301:1646–1656
- Myers RR, Murakami H, Powell HC (1986) Reduced nerve blood flow in edematous neuropathies: a biomechanical mechanism. *Microvasc Res* 32:145–151
- Nishida Y, Yamada Y, Kanemaru H, Ohazama A, Maeda T, Seo K (2018) Vascularization via activation of VEGF-VEGFR signaling is essential for peripheral nerve regeneration. *Biomed Res* 39:287–294
- Nomi M, Atala A, Coppi PD, Soker S (2002) Principles of neovascularization for tissue engineering. *Mol Asp Med* 23:463–483
- Nukada H (1988) Post-traumatic endoneurial neovascularization and nerve regeneration: a morphometric study. *Brain Res* 449:89–96
- Nukada H, Dyck PJ (1984) Microsphere embolization of nerve capillaries and fiber degeneration. *Am J Pathol* 115:275–287
- Nukada H, Dyck PJ (1986) Neovascularization after ischemic nerve injury. *Exp Neurol* 92:391–397
- Nukada H, Dyck PJ, Karnes JL (1985) Spatial distribution of capillaries in rat nerves: correlation to ischemic damage. *Exp Neurol* 87:369–376
- Nukada H, van Rij AM, Packer SG, McMorran PD (1996) Pathology of acute and chronic ischaemic neuropathy in atherosclerotic peripheral vascular disease. *Brain* 119(Pt 5): 1449–1460
- O'Brien JP, Mackinnon SE, MacLean AR, Hudson AR, Dellon AL, Hunter DA (1987) A model of chronic nerve compression in the rat. *Ann Plast Surg* 19:430–435
- O'Rourke C, Drake RA, Cameron GW, Loughlin AJ, Phillips JB (2015) Optimising contraction and alignment of cellular collagen hydrogels to achieve reliable and consistent engineered anisotropic tissue. *J Biomater Appl* 30:599–607
- O'Rourke C, Day AGE, Murray-Dunning C, Thanabalasundaram L, Cowan J, Stevanato L, Grace N, Cameron G, Drake RAL, Sinden J, Phillips JB (2018) An allogeneic “off the shelf” therapeutic strategy for peripheral nerve tissue engineering using clinical grade human neural stem cells. *Sci Rep* 8:2951
- Ochoa J, Fowler TJ, Gilliatt RW (1972) Anatomical changes in peripheral nerves compressed by a pneumatic tourniquet. *J Anat* 113:433–455
- Oh WJ, Gu C (2013) Establishment of neurovascular congruency in the mouse whisker system by an independent patterning mechanism. *Neuron* 80:458–469
- Ohana M, Moser T, Moussaoui A, Kremer S, Carlier RY, Liverneaux P, Dietemann JL (2014) Current and future imaging of the peripheral nervous system. *Diagn Interv Imaging* 95:17–26
- Olsson Y, Reese TS (1971) Permeability of vasa nervorum and perineurium in mouse sciatic nerve studied by fluorescence and electron microscopy. *J Neuropathol Exp Neurol* 30:105–119
- Oudega M, Gautier SE, Chapon P, Frago M, Bates ML, Parel JM, Bunge MB (2001) Axonal regeneration into Schwann cell grafts within resorbable poly(alpha-hydroxyacid) guidance channels in the adult rat spinal cord. *Biomaterials* 22:1125–1136

- Ozcan G, Shenaq S, Mirabi B, Spira M (1993a) Nerve regeneration in a bony bed: vascularized versus nonvascularized nerve grafts. *Plast Reconstr Surg* 91:1322–1331
- Ozcan G, Shenaq S, Spira M (1993b) Vascularized nerve tube: an experimental alternative for vascularized nerve grafts over short gaps. *J Reconstr Microsurg* 9:405–413
- Parrinello S, Napoli I, Ribeiro S, Wingfield Digby P, Fedorova M, Parkinson DB, Doddrell RD, Nakayama M, Adams RH, Lloyd AC (2010) EphB signaling directs peripheral nerve regeneration through Sox2-dependent Schwann cell sorting. *Cell* 143:145–155
- Penkert G, Bini W, Samii M (1988) Revascularization of nerve grafts: an experimental study. *J Reconstr Microsurg* 4:319–325
- Penna V, Munder B, Stark GB, Lang EM (2011) An in vivo engineered nerve conduit – fabrication and experimental study in rats. *Microsurgery* 31:395–400
- Pereira Lopes FR, Lisboa BC, Frattini F, Almeida FM, Tomaz MA, Matsumoto PK, Langone F, Lora S, Melo PA, Borojevic R, Han SW, Martinez AM (2011) Enhancement of sciatic nerve regeneration after vascular endothelial growth factor (VEGF) gene therapy. *Neuropathol Appl Neurobiol* 37:600–612
- Petrova ES, Isaeva EN, Kolos EA, Korzhevskii DE (2018) Vascularization of the damaged nerve under the effect of experimental cell therapy. *Bull Exp Biol Med* 165:161–165
- Pho RW, Lee YS, Rujiwetpongstorn V, Pang M (1985) Histological studies of vascularised nerve graft and conventional nerve graft. *J Hand Surg Br* 10:45–48
- Podhajsky RJ, Myers RR (1993) The vascular response to nerve crush: relationship to Wallerian degeneration and regeneration. *Brain Res* 623:117–123
- Podhajsky RJ, Myers RR (1994) The vascular response to nerve transection: neovascularization in the silicone nerve regeneration chamber. *Brain Res* 662:88–94
- Poduslo JF, Curran GL (1992) Increased permeability across the blood-nerve barrier of albumin glycated in vitro and in vivo from patients with diabetic polyneuropathy. *Proc Natl Acad Sci U S A* 89:2218–2222
- Poduslo JF, Curran GL, Dyck PJ (1988) Increase in albumin, IgG, and IgM blood-nerve barrier indices in human diabetic neuropathy. *Proc Natl Acad Sci U S A* 85:4879–4883
- Poduslo JF, Curran GL, Berg CT (1994) Macromolecular permeability across the blood-nerve and blood-brain barriers. *Proc Natl Acad Sci U S A* 91(12):5705–5709
- Poduslo JF, Curran GL, Gill JS (1998) Putrescine-modified nerve growth factor: bioactivity, plasma pharmacokinetics, blood-brain/nerve barrier permeability, and nervous system biodistribution. *J Neurochem* 71:1651–1660
- Pugliese G, Tilton RG, Speedy A, Chang K, Santarelli E, Province MA, Eades D, Sherman WR, Williamson JR (1989) Effects of very mild versus overt diabetes on vascular haemodynamics and barrier function in rats. *Diabetologia* 32:845–857
- Quénu J, Lejars F (1894) Études sur la système ciculatoire. G. Steinheil (ed.) Paris
- Ramos T, Ahmed M, Wieringa P, Moroni L (2015) Schwann cells promote endothelial cell migration. *Cell Adhes Migr* 9:441–451
- Ray WZ, Mackinnon SE (2010) Management of nerve gaps: autografts, allografts, nerve transfers, and end-to-side neurorrhaphy. *Exp Neurol* 223:77–85
- Rbia N, Bulstra LF, Thaler R, Hovius SER, van Wijnen AJ, Shin AY (2019) In vivo survival of mesenchymal stromal cell-enhanced decellularized nerve grafts for segmental peripheral nerve reconstruction. *J Hand Surg Am* 44:514.e1–514.e11
- Rechthand E, Hervonen A, Sato S, Rapoport SI (1986) Distribution of adrenergic innervation of blood vessels in peripheral nerve. *Brain Res* 374:185–189
- Rechthand E, Smith QR, Rapoport SI (1987) Transfer of nonelectrolytes from blood into peripheral nerve endoneurium. *Am J Physiol Heart Circ Physiol* 252(6):H1175–H1182
- Rechthand E, Sato S, Oberg PA, Rapoport SI (1988) Sciatic nerve blood flow response to carbon dioxide. *Brain Res* 446:61–66
- Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, Pell CL, Johnstone BH, Considine RV, March KL (2004) Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* 109:1292–1298

- Reinhold AK, Rittner HL (2020) Characteristics of the nerve barrier and the blood dorsal root ganglion barrier in health and disease. *Exp Neurol* 327:113244
- Restrepo Y, Merle M, Michon J, Folliguet B, Barrat E (1985) Free vascularized nerve grafts: an experimental study in the rabbit. *Microsurgery* 6:78–84
- Reynolds DL Jr, Jacobson JA, Inampudi P, Jamadar DA, Ebrahim FS, Hayes CW (2004) Sonographic characteristics of peripheral nerve sheath tumors. *AJR Am J Roentgenol* 182:741–744
- Ritchie JM (1967) The oxygen consumption of mammalian non-myelinated nerve fibres at rest and during activity. *J Physiol* 188:309–329
- Ronchi G, Cillino M, Gambarotta G, Fornasari BE, Raimondo S, Pugliese P, Tos P, Cordova A, Moschella F, Geuna S (2017) Irreversible changes occurring in long-term denervated Schwann cells affect delayed nerve repair. *J Neurosurg* 127:843–856
- Rose EH, Kowalski TA, Norris MS (1989) The reversed venous arterialized nerve graft in digital nerve reconstruction across scarred beds. *Plast Reconstr Surg* 83:593–604
- Rouwkema J, Koopman B, Blitterswijk C, Dhert W, Malda J (2010) Supply of nutrients to cells in engineered tissues. *Biotechnol Bioeng* 26:163–178
- Rundquist I, Smith QR, Michel ME, Ask P, Oberg PA, Rapoport SI (1985) Sciatic nerve blood flow measured by laser Doppler flowmetry and [¹⁴C]iodoantipyrine. *Am J Physiol* 248: H311–H317
- Saffari TM, Badreldin A, Mathot F, Bagheri L, Bishop AT, van Wijnen AJ, Shin AY (2020a) Surgical angiogenesis modifies the cellular environment of processed nerve allografts in a rat sciatic nerve defect model. *Gene* 751:144711
- Saffari TM, Bedar M, Hundepool CA, Bishop AT, Shin AY (2020b) The role of vascularization in nerve regeneration of nerve graft. *Neural Regen Res* 15:1573–1579
- Saffari TM, Mathot F, Friedrich PF, Bishop AT, Shin AY (2020c) Revascularization patterns of nerve allografts in a rat sciatic nerve defect model. *J Plast Reconstr Aesthet Surg* 73:460–468
- Saffari TM, Mathot F, Thaler R, van Wijnen AJ, Bishop AT, Shin AY (2020d) Microcomputed analysis of nerve angioarchitecture after combined stem cell delivery and surgical angiogenesis to nerve allograft. *J Plast Reconstr Aesthet Surg* 74(8):1919–1930
- Saffari S, Saffari TM, Ulrich DJO, Hovius SER, Shin AY (2021) The interaction of stem cells and vascularity in peripheral nerve regeneration. *Neural Regen Res* 16:1510–1517
- Sakuma M, Gorski G, Sheu SH, Lee S, Barrett LB, Singh B, Omura T, Latremoliere A, Woolf CJ (2016) Lack of motor recovery after prolonged denervation of the neuromuscular junction is not due to regenerative failure. *Eur J Neurosci* 43:451–462
- Sanen K, Martens W, Georgiou M, Ameloot M, Lambrechts I, Phillips J (2017) Engineered neural tissue with Schwann cell differentiated human dental pulp stem cells: potential for peripheral nerve repair? *J Tissue Eng Regen Med* 11:3362–3372
- Saray A, Teoman Tellioglu A, Altinok G (2002) Prefabrication of a free peripheral nerve graft following implantation on an arteriovenous pedicle. *J Reconstr Microsurg* 18:281–288
- Sarhane KA, Slavin BR, Hricz N, Malapati H, Guo YN, Grzelak M, Chang IA, Shappell H, von Guionneau N, Wong AL, Mi R, Höke A, Tuffaha SH (2021) Defining the relative impact of muscle versus Schwann cell denervation on functional recovery after delayed nerve repair. *Exp Neurol* 339:113650
- Sarker MD, Naghieh S, McInnes AD, Schreyer DJ, Chen X (2018) Regeneration of peripheral nerves by nerve guidance conduits: influence of design, biopolymers, cells, growth factors, and physical stimuli. *Prog Neurobiol* 171:125–150
- Saska S, Pilatti L, Blay A, Shibli JA (2021) Bioresorbable polymers: advanced materials and 4D printing for tissue engineering. *Polymers* 13:563
- Scallan JP, Hill MA, Davis MJ (2015) Lymphatic vascular integrity is disrupted in type 2 diabetes due to impaired nitric oxide signalling. *Cardiovasc Res* 107:89–97
- Schlau M, Terheyden-Keighley D, Theis V, Mannherz HG, Theiss C (2018) VEGF triggers the activation of cofilin and the Arp2/3 complex within the growth cone. *Int J Mol Sci* 19:384
- Schmelzer JD, Zochodne DW, Low PA (1989) Ischemic and reperfusion injury of rat peripheral nerve. *Proc Natl Acad Sci U S A* 86:1639–1642

- Seckel BR, Ryan SE, Simons JE, Gagne RG, Watkins E Jr (1986) Vascularized versus non-vascularized nerve grafts: an experimental structural comparison. *Plast Reconstr Surg* 78:211–220
- Seddon H (1975) *Surgical disorders of the peripheral nerves*. Churchill Livingstone, Edinburgh/London
- Segarra M, Kirchmaier BC, Acker-Palmer A (2015) A vascular perspective on neuronal migration. *Mech Dev* 138(Pt 1):17–25
- Segarra M, Aburto MR, Hefendehl J, Acker-Palmer A (2019) Neurovascular interactions in the nervous system. *Annu Rev Cell Dev Biol* 35:615–635
- Seiler JG III, Milek MA, Carpenter GK, Swionkowski MF (1989) Intraoperative assessment of median nerve blood flow during carpal tunnel release with laser Doppler flowmetry. *J Hand Surg Am* 14:986–991
- Selander D, Månsson LG, Karlsson L, Svanvik J (1985) Adrenergic vasoconstriction in peripheral nerves of the rabbit. *Anesthesiology* 62:6–10
- Selim OA, Lakhani S, Midha S, Mosahebi A, Kalaskar DM (2021) Three-dimensional engineered peripheral nerve: toward a new era of patient-specific nerve repair solutions. *Tissue Eng Part B Rev*. <https://doi.org/10.1089/ten.TEB.2020.0355>. Epub ahead of print. PMID: 33593147
- Shafi M, Hattori Y, Doi K (2010) Surgical technique of harvesting vascularized superficial radial nerve graft. *J Hand Surg Am* 35:312–315
- Shdanow D (1931) Die Lymphwege des peripherischen und Zentralnervensystems: I Die abführenden Lymphgefäße von Nervenstämmen der Extremitäten des Menschen. *Anat Anz* 71:231–245
- Shibata M, Tsai TM, Firrell J, Breidenbach WC (1988) Experimental comparison of vascularized and nonvascularized nerve grafting. *J Hand Surg Am* 13:358–365
- Shupeck M, Ward KK, Schmelzer JD, Low PA (1989) Comparison of nerve regeneration in vascularized and conventional grafts: nerve electrophysiology, norepinephrine, prostacyclin, malondialdehyde, and the blood-nerve barrier. *Brain Res* 493:225–230
- Sladky JT, Greenberg JH, Brown MJ (1985) Regional perfusion in normal and ischemic rat sciatic nerves. *Ann Neurol* 17:191–195
- Slutsky DJ (2013) Vascularized ulnar nerve graft. In: *The art of microsurgical hand reconstruction*. Georg Thieme Verlag, Stuttgart
- Smith DR, Kobrine AI, Rizzoli HV (1977) Absence of autoregulation in peripheral nerve blood flow. *J Neurol Sci* 33:347–352
- Soman SS, Vijayavenkataraman S (2020) Perspectives on 3D bioprinting of peripheral nerve conduits. *Int J Mol Sci* 21:5792
- Sommer C, Myers RR (1996) Vascular pathology in CCI neuropathy: a quantitative temporal study. *Exp Neurol* 141:113–119
- Sonabend AM, Smith P, Huang JH, Winfree C (2012) Peripheral nerve injury. In: *Schmidek and sweet operative neurosurgical techniques: indications, methods, and results: sixth edition*, vol 2. Elsevier, Amsterdam, pp 2225–2238
- Sondell M, Lundborg G, Kanje M (1999) Vascular endothelial growth factor stimulates Schwann cell invasion and neovascularization of acellular nerve grafts. *Brain Res* 846:219–228
- Staniforth P, Fisher TR (1978) The effects of sural nerve excision in autogenous nerve grafting. *Hand* 10:187–190
- Stanton-Hicks M (2018) Anatomy and physiology related to peripheral nerve stimulation. *Neuromodulation* 24:723–727
- Stoll G, Wilder-Smith E, Bendszus M (2013) Imaging of the peripheral nervous system. *Handb Clin Neurol* 115:137–153
- Strange FG (1947) An operation for nerve pedicle grafting; preliminary communication. *Br J Surg* 34:423–425
- Stubbs D, Deproto J, Nie K, Englund C, Mahmud I, Hevner R, Molnár Z (2009) Neurovascular congruence during cerebral cortical development. *Cereb Cortex* 19(Suppl 1):i32–i41
- Sunderland S (1945a) Blood supply of peripheral nerves; practical considerations. *Arch Neurol Psychiatr* 54:280–282

- Sunderland S (1945b) Blood supply of the nerves of the upper limb in man. *Arch Neurol Psychiatr* 53:91–115
- Sunderland S (1945c) Blood supply of the sciatic nerve and its popliteal divisions in man. *Arch Neurol Psychiatr* 54:283–289
- Sunderland S (1951) A classification of peripheral nerve injuries producing loss of function. *Brain* 74:491–516
- Sunderland S (1965) The connective tissues of peripheral nerves. *Brain* 88:841–854
- Sunderland S (1978) Nerves and nerve injuries, 2nd edn. Churchill Livingstone, Edinburgh/London/New York, p 1046
- Sunderland S (1991) Nerve injuries and their repair: a critical appraisal. Churchill Livingstone, Edinburgh/London
- Sweat RS, Sloas DC, Murfee WL (2014) VEGF-C induces lymphangiogenesis and angiogenesis in the rat mesentery culture model. *Microcirculation* 21:532–540
- Takahashi K, Nomura S, Tomita K, Matsumoto T (1988) Effects of peripheral nerve stimulation on the blood flow of the spinal cord and the nerve root. *Spine* 13:1278–1283
- Takahashi Y, Sipp D, Enomoto H (2013) Tissue interactions in neural crest cell development and disease. *Science* 341:860–863
- Takamatsu H, Takegahara N, Nakagawa Y, Tomura M, Taniguchi M, Friedel RH, Rayburn H, Tessier-Lavigne M, Yoshida Y, Okuno T, Mizui M, Kang S, Nojima S, Tsujimura T, Nakatsuji Y, Katayama I, Toyofuku T, Kikutani H, Kumanogoh A (2010) Semaphorins guide the entry of dendritic cells into the lymphatics by activating myosin II. *Nat Immunol* 11:594–600
- Tarlov IM, Epstein JA (1945) Nerve grafts: the importance of an adequate blood supply. *J Neurosurg* 2:49–71
- Taylor GI (1978) Nerve grafting with simultaneous microvascular reconstruction. *Clin Orthop Relat Res*:56–70
- Taylor GI, Daniel RK (1973) The free flap: composite tissue transfer by vascular anastomosis. *Aust N Z J Surg* 43:1–3
- Taylor GI, Ham FJ (1976) The free vascularized nerve graft. A further experimental and clinical application of microvascular techniques. *Plast Reconstr Surg* 57:413–426
- Terzis JK, Kostopoulos VK (2009) Vascularized ulnar nerve graft: 151 reconstructions for post-traumatic brachial plexus palsy. *Plast Reconstr Surg* 123:1276–1291
- Terzis JK, Kostopoulos VK (2010a) Vascularized nerve grafts and vascularized fascia for upper extremity nerve reconstruction. *Hand* 5:19–30
- Terzis JK, Kostopoulos VK (2010b) Vascularized nerve grafts for lower extremity nerve reconstruction. *Ann Plast Surg* 64:169–176
- Terzis JK, Skoulis TG, Soucacos PN (1995) Vascularized nerve grafts. A review. *Int Angiol* 14:264–277
- Theriault M, Dort J, Sutherland G, Zochodne DW (1997) Local human sural nerve blood flow in diabetic and other polyneuropathies. *Brain* 120(Pt 7):1131–1138
- Thiele FH, Embleton HD (1914) Royal Society of Medicine – Section of Pathology. *Lancet* 183:609–612
- Thompson N, Ravagli E, Mastitskaya S, Iacoviello F, Aristovich K, Perkins J, Shearing PR, Holder D (2020) MicroCT optimisation for imaging fascicular anatomy in peripheral nerves. *J Neurosci Methods* 338:108652
- Thomsen K, Rubin I, Lauritzen M (2000) In vivo mechanisms of acetylcholine-induced vasodilation in rat sciatic nerve. *Am J Physiol Heart Circ Physiol* 279:H1044–H1054
- Tilton RG, Chang K, Nyengaard JR, Van den Enden M, Ido Y, Williamson JR (1995) Inhibition of sorbitol dehydrogenase. Effects on vascular and neural dysfunction in streptozocin-induced diabetic rats. *Diabetes* 44:234–242
- Townsend PL, Taylor GI (1984) Vascularised nerve grafts using composite arterialised neuro-venous systems. *Br J Plast Surg* 37:1–17
- Ubogu EE (2020) Biology of the human blood-nerve barrier in health and disease. *Exp Neurol* 328:113272

- Ugrenovic SZ, Jovanovic ID, Kovacevic P, Petrović S, Simic T (2013) Similarities and dissimilarities of the blood supplies of the human sciatic, tibial, and common peroneal nerves. *Clin Anat* 26:875–882
- Vallat JM, Leboutet MJ, Loubet A, Hugon J, Moreau JJ (1988) Effects of glycerol injection into rat sciatic nerve. *Muscle Nerve* 11:540–545
- Volpi N, Agliano M, Massai L, Alessandrini C, Rossi S, Pucci AM, Grasso G (2006) Lymphatic vessels in human sural nerve: immunohistochemical detection by D2-40. *Lymphology* 39:171–173
- Volpi N, Guarna M, Lorenzoni P, Franci D, Massai L, Grasso G (2013) Characterization of lymphatic vessels in human peripheral neuropathies. *Ital J Anat Embryol* 117(2):12
- Watt SM, Gullo F, van der Garde M, Markeson D, Camicia R, Khoo CP, Zwaginga JJ (2013) The angiogenic properties of mesenchymal stem/stromal cells and their therapeutic potential. *Br Med Bull* 108:25–53
- Weddell G (1942) Axonal regeneration in cutaneous nerve plexuses. *J Anat* 77:49–62.3
- Weerasuriya A (1988) Patterns of change in endoneurial capillary permeability and vascular space during Wallerian degeneration. *Brain Res* 445:181–187
- Weerasuriya A (1990) Patterns of change in endoneurial capillary permeability and vascular space during nerve regeneration. *Brain Res* 510:135–139
- Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE (2001) Soft lithography in biology and biochemistry. *Annu Rev Biomed Eng* 3:335–373
- Wiegand KA, Capitosti SM, Anderson CR, Price RJ, Blackman BR, Brown ML, Botchwey EA (2006) Small molecule inducers of angiogenesis for tissue engineering. *Tissue Eng* 12:1903–1913
- Wolford LM, Stevao EL (2003) Considerations in nerve repair. *Proceedings* 16:152–156
- Wongtrakul S, Bishop AT, Friedrich PF (2002) Vascular endothelial growth factor promotion of neoangiogenesis in conventional nerve grafts. *J Hand Surg Am* 27:277–285
- Wu P, Tong Z, Luo L, Zhao Y, Chen F, Li Y, Huselstein C, Ye Q, Chen Y (2021) Comprehensive strategy of conduit guidance combined with VEGF producing Schwann cells accelerates peripheral nerve repair. *Bioact Mater* 6:3515–3527
- Xia B, Lv Y (2018) Dual-delivery of VEGF and NGF by emulsion electrospun nanofibrous scaffold for peripheral nerve regeneration. *Mater Sci Eng C Mater Biol Appl* 82:253–264
- Xu QG, Zochodne DW (2002) Ischemia and failed regeneration in chronic experimental neuromas. *Brain Res* 946:24–30
- Xu Q, Midha R, Zochodne DW (2010) The microvascular impact of focal nerve trunk injury. *J Neurotrauma* 27:639–646
- Yan L, Liu S, Qi J, Zhang Z, Zhong J, Li Q, Liu X, Zhu Q, Yao Z, Lu Y, Gu L (2019) Three-dimensional reconstruction of internal fascicles and microvascular structures of human peripheral nerves. *Int J Numer Method Biomed Eng* 35:e3245
- Yapici AK, Bayram Y, Akgun H, Gumus R, Zor F (2017) The effect of in vivo created vascularized neurotube on peripheral nerve regeneration. *Injury* 48:1486–1491
- Yi S, Zhang Y, Gu X, Huang L, Zhang K, Qian T, Gu X (2020) Application of stem cells in peripheral nerve regeneration. *Burns Trauma* 8:tkaa002
- Yorek MA (2015) Vascular impairment of epineurial arterioles of the sciatic nerve: implications for diabetic peripheral neuropathy. *Rev Diabet Stud* 12:13–28
- Zadegan SA, Firouzi M, Nabian MH, Zanjani LO, Ashtiani AM, Kamrani RS (2015a) Two-stage nerve graft in severe scar: a time-course study in a rat model. *Arch Bone Jt Surg* 3:82–87
- Zadegan SA, Firouzi M, Nabian MH, Zanjani LO, Kamrani RS (2015b) Two-stage nerve graft using a silicone tube. *Front Surg* 2:12
- Zhang RC, Du WQ, Zhang JY, Yu SX, Lu FZ, Ding HM, Cheng YB, Ren C, Geng DQ (2021a) Mesenchymal stem cell treatment for peripheral nerve injury: a narrative review. *Neural Regen Res* 16:2170–2176
- Zhang Y, Kumar P, Lv S, Xiong D, Zhao H, Cai Z, Zhao X (2021b) Recent advances in 3D bioprinting of vascularized tissues. *Mater Des* 199:109398

- Zhao Z, Wang Y, Peng J, Ren Z, Zhan S, Liu Y, Zhao B, Zhao Q, Zhang L, Guo Q, Xu W, Lu S (2011) Repair of nerve defect with acellular nerve graft supplemented by bone marrow stromal cells in mice. *Microsurgery* 31:388–394
- Zhao B, Zhao Z, Ma J, Ma X (2019) Modulation of angiogenic potential of tissue-engineered peripheral nerve by covalent incorporation of heparin and loading with vascular endothelial growth factor. *Neurosci Lett* 705:259–264
- Zochodne DW (2002) Nerve and ganglion blood flow in diabetes: an appraisal. *Int Rev Neurobiol* 50:161–202
- Zochodne DW (2018) Local blood flow in peripheral nerves and their ganglia: resurrecting key ideas around its measurement and significance. *Muscle Nerve* 57:884–895
- Zochodne DW, Ho LT (1990) Endoneurial microenvironment and acute nerve crush injury in the rat sciatic nerve. *Brain Res* 535:43–48
- Zochodne DW, Ho LT (1991a) Influence of perivascular peptides on endoneurial blood flow and microvascular resistance in the sciatic nerve of the rat. *J Physiol* 444:615–630
- Zochodne DW, Ho LT (1991b) Stimulation-induced peripheral nerve hyperemia: mediation by fibers innervating vasa nervorum? *Brain Res* 546:113–118
- Zochodne DW, Ho LT (1992a) Hyperemia of injured peripheral nerve: sensitivity to CGRP antagonism. *Brain Res* 598:59–66
- Zochodne DW, Ho LT (1992b) The influence of indomethacin and guanethidine on experimental streptozotocin diabetic neuropathy. *Can J Neurol Sci* 19:433–441
- Zochodne DW, Ho LT (1992c) Normal blood flow but lower oxygen tension in diabetes of young rats: microenvironment and the influence of sympathectomy. *Can J Physiol Pharmacol* 70:651–659
- Zochodne DW, Low PA (1990) Adrenergic control of nerve blood flow. *Exp Neurol* 109:300–307
- Zochodne DW, Nguyen C (1997) Angiogenesis at the site of neuroma formation in transected peripheral nerve. *J Anat* 191(Pt 1):23–30
- Zochodne DW, Nguyen C (1999) Increased peripheral nerve microvessels in early experimental diabetic neuropathy: quantitative studies of nerve and dorsal root ganglia. *J Neurol Sci* 166:40–46
- Zochodne DW, Low PA, Dyck PJ (1989) Adrenergic sympathectomy ablates unmyelinated fibers in the rat “preganglionic” cervical sympathetic trunk. *Brain Res* 498:221–228
- Zochodne DW, Huang ZX, Ward KK, Low PA (1990) Guanethidine-induced adrenergic sympathectomy augments endoneurial perfusion and lowers endoneurial microvascular resistance. *Brain Res* 519:112–117
- Zochodne DW, Ho LT, Gross PM (1992) Acute endoneurial ischemia induced by epineurial endothelin in the rat sciatic nerve. *Am J Physiol* 263:H1806–H1810
- Zochodne DW, Allison JA, Ho W, Ho LT, Hargreaves K, Sharkey KA (1995) Evidence for CGRP accumulation and activity in experimental neuromas. *Am J Physiol* 268:H584–H590
- Zochodne DW, Levy D, Zwiers H, Sun H, Rubin I, Cheng C, Lauritzen M (1999) Evidence for nitric oxide and nitric oxide synthase activity in proximal stumps of transected peripheral nerves. *Neuroscience* 91:1515–1527

The Immune Response and Implications for Nerve Repair

Victoria H. Robertson

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Abstract

This chapter aims to provide an overview of the host response to allografts or tissue-engineered constructs containing allogeneic cells in peripheral nerve repair, and potential approaches for promoting the survival of nerve grafts. A large body of current research aims to improve surgical approaches for nerve repair beyond that of the autograft. Potential approaches include nerve allografts, or tissue-engineered nerve constructs which could provide an unlimited source of

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donor tissue for nerve injury repair. This field requires an interdisciplinary approach to the design of novel therapies, and consideration of the immune response to transplants should not be overlooked. There are many benefits of including living donor cells within transplanted nerve conduits which can improve axonal guidance, vascularization, and promote the release of growth factors to increase regeneration. It is most likely that donor cells or tissues will be of allogeneic origin, with associated implications for eliciting an immune response on transplantation. The following sections provide a summary of the immune response to nerve grafts for consideration in the development of approaches to nerve repair, including some approaches currently under investigation for preventing rejection of transplants.

1 Introduction

Injuries to the peripheral nerves occur frequently as a result of trauma and can have a life-changing impact on individuals, leaving them with disabling motor and sensory deficits. Successful recovery requires the development of a number of cellular and molecular processes distal to the lesion site and toward the denervated target known as Wallerian degeneration (Waller 1850; Rotshenker 2011). After degeneration of detached distal axons, myelin and axonal debris are broken down and phagocytosed by Schwann cells (SCs). These SCs secrete cytokines and chemokines which recruit immune cells into the injured nerve, particularly macrophages, which contribute to myelin clearance and release growth factors leading to the proliferation of SCs and fibroblasts. SCs first degenerate and begin to upregulate regeneration-associated genes. Proliferating SCs then align to form bands of Büngner and provide conduits for regenerating axons (Stoll et al. 1989). In the distal nerve stump, SCs also secrete extracellular matrix (ECM) molecules, such as laminin, and trophic factors to promote axon growth.

The management of injuries depends on various factors, including the type and location of injury, the size of the nerve deficit, the timing of injury, and any associated soft tissue injury (Palispis and Gupta 2017). Total disruption of the peripheral nerve (neurotmesis) requires surgical realignment of the nerve stumps, with the primary aim of allowing reinnervation of target organs through guidance of regenerating axons into the distal nerve. Direct epineurial repair is suitable when tension-free coaptation and gross fascicular matching of the proximal and distal nerve ends can be achieved (Grinsell and Keating 2014). Although surgical advances in peripheral nerve repair have been developed following research into the biology of nerve injury, only partial improvement is still achieved (discussed in chapter ► “The History of Nerve Repair”). Sensory recovery has been reported in just 42% of digital nerve repairs, and only 25% with excellent outcomes (Paprottka et al. 2013). With delayed repair following injury, over time retraction of the stumps will cause an increased gap length for repair, meaning primary suturing of the nerve stumps will cause tension at the coaptation site (Siemionow and Sonmez 2007). Repair of the nerve gap therefore requires bridging to allow nerve regeneration and

restoration of function while avoiding causing tension from direct apposition of the cut ends. Novel strategies for bridging this nerve gap are currently under investigation in order to provide a greater source of potential donor nerve, and improve functional outcomes after repair, through tissue engineering and the use of bio-materials. A number of these strategies require the transplantation of allografts or engineered nerve constructs containing allogeneic cells. While potentially resolving the issues of tissue supply and increasing regenerative potential, this approach may cause immunological rejection of transplanted cells or tissues; thus, a consideration of the host immune response in nerve repair is essential to enable survival and function of implanted tissues to develop feasible approaches for translation. This chapter will discuss the immune response to transplants in the context of nerve repair, and potential approaches to avoid this issue. An additional important consideration in the preparation of engineered tissues for transplantation is the response to the implanted material itself. The foreign body response to implanted materials is discussed briefly but is reviewed more thoroughly elsewhere (Anderson et al. 2008). Thus, the main focus of this chapter will be on the immune response to allo-transplanted cells or tissues.

2 Peripheral Nerve Repair Strategies

Currently, a nerve autograft derived from a different donor site of the patient is the gold standard for clinical repair of nerve gap injuries (Ijkema-Paassen et al. 2004). The sural nerve is a common donor source as it is easily obtained and can be sacrificed for the repair and reinnervation of more critical muscle. However, despite successful outcomes of the nerve autograft, sacrificing a donor nerve from the patient creates damage in an otherwise healthy area including scarring, sensory loss, and the risk of neuroma formation (Battiston et al. 2017). In addition to this donor site morbidity, the repair of large defects requires more donor nerve than may be available as autologous grafts. Size and fascicular matching for autografts is a challenge, and the procedure and injury itself can result in scarring and fibrosis leading to poor regeneration (Ijkema-Paassen et al. 2004). Clinically, it is estimated that there is a 50% loss of axons at each coaptation site, resulting in successful regeneration of 50% of the original axons through the repair site in primary nerve repair. In a nerve graft with two coaptation sites, this will reduce to 25% successful regeneration through the graft, decreasing further depending on other factors, including the distance to the target (Grinsell and Keating 2014).

The disadvantages associated with the use of autografts demanded the development of new techniques to provide “off-the-shelf” alternatives to serve as axonal guidance channels. The acellular nerve graft provides an alternative ECM scaffold which can be repopulated by host cells to promote regeneration (Pan et al. 2019). Evidence has shown successful regeneration across a nerve gap in a rat sciatic nerve injury model (Kim et al. 2004). There is also evidence for successful regeneration across short gaps (3 cm or below) in patients with digital nerve injuries, following implantation of bioabsorbable polyglycolic acid tubes (Mackinnon and Dellon

1990). Such acellular allogeneic nerve grafts have been approved for clinical use, including Avance[®], produced by AxoGen, Inc. However, although this product showed greater efficacy than a Type 1 collagen guidance conduit (NeuraGen[®] nerve guide), it did not yield as good regeneration as an isograft when tested in vivo (Whitlock et al. 2009). The authors concluded that, in a larger gap model, the decellularized allograft may not be able to produce the same results as a nerve isograft; however, it may be suitable for smaller gaps (Whitlock et al. 2009). Additional approved guidance conduits are mostly fabricated from Type 1 collagen and suitable for small nerve defects, including NeuraGen, NeuroMatrix, NeuroFlex, NeuraWrap, and NeuroMend (discussed in chapter ► [“Biomaterials and Scaffolds for Repair of the Peripheral Nervous System”](#)). Outcomes following transplantation of these conduits show functional improvement and regeneration, though for long gap injuries (>3 cm), regeneration can be inadequate for recovery and the outcomes following autograft remain superior to nerve conduits (Grinsell and Keating 2014; Pan et al. 2019).

The use of cellular peripheral nerve allografts in the place of autografts would offer an abundant source of nerve for transplantation from cadaveric donors. Allografts can act as viable conduits for transplantation, allowing host sensory and motor axons to successfully grow and reach their targets (Moore et al. 2009). Allografting also offers the potential for transplantation of the same nerve type, for example, replacing mixed sensory-motor nerves with the same donor nerve to improve recovery. Commonly, the sural nerve (sensory) is used as donor nerve for peripheral nerve autografts, when the nerve being repaired may be motor. Studies in rodents and patients with nerve injury have shown that, unlike autografts, allografts will be rejected by the host immune system. As immunological recognition is due to the recognition of foreign histocompatibility antigens on donor cells within the graft, decellularization of the nerve prior to implantation is possible to provide a conduit along which axonal regeneration can occur. This can be achieved through irradiation, freeze thawing, detergent-processing, and cold preservation (Lin et al. 2013). However, without the presence of therapeutic SCs within the graft, regeneration is greatly reduced. In addition, there are currently limited options available, with decellularized allografts presently being offered at such a high cost that, despite the associated negatives, the peripheral nerve autograft is still the preferred option. An alternative method to promote the acceptance of a nerve allograft is the use of immunosuppressant drugs, and the use of cadaveric nerve allografts has been explored to restore nerve continuity clinically (Mackinnon et al. 2001). Immunosuppression for the prevention of allograft rejection requires prolonged systemic administration and can result in significant side effects including nephrotoxicity and hepatotoxicity (Brenner et al. 2002; Rezzani 2006; Teh et al. 2011). As peripheral nerve injury (PNI) is not in itself life-threatening, such severe side effects preclude the use of systemic immunosuppression in these patients, thus removing the potential for the use of nerve allografts as a source of donor tissue.

An alternative to the grafting of donor nerve is the development of engineered nerve tissues for transplantation. The potential of this approach could increase both supply and therapeutic efficacy, as tissues could be developed incorporating

combinations of therapeutic cells, growth factors, and materials specifically designed to improve regeneration. An “ideal conduit” is deemed to have the following requirements: (1) supply a biocompatible or biodegradable tube for integration into the surrounding tissues while supporting axonal regeneration and progression; (2) be of a size and length adequate to connect the stumps of the damaged nerve; (3) contain throughout its length substances exerting chemotactic attraction and allowing axonal progression; and (4) protect regenerating axons against scar invasion (Brunelli et al. 1994). Commercially available devices have included hollow tubes manufactured from biodegradable polymer/collagen, though their efficacy is restricted to short defects and functional recovery is limited and does not surpass that of autologous nerve grafts (Pabari et al. 2014). A large body of research has sought to develop tissue-engineered constructs for nerve repair (Pabari et al. 2014; Faroni et al. 2015; Carvalho et al. 2019; Raza et al. 2020), though there has not yet been a substantial translation to the clinic. The incorporation of therapeutic cells into tissue-engineered constructs is considered to be an important factor in improving regeneration, and a number of options have been tested including SCs, neural stem cells, embryonic stem cells, and bone marrow stromal cells (Carvalho et al. 2019). As these may be of allogeneic origin, the use of mature cells poses issues of immunological rejection. The development of off-the-shelf acellular products therefore provides an easier route to translation, overcoming issues with variability due to the incorporation of cell products, and regulatory approval of these novel therapeutics. Developing methods to circumvent the problems of immunological rejection would increase the feasibility of translating cell-seeded nerve guidance conduits for peripheral nerve repair.

3 Mechanisms of Rejection

The function of the immune system is to protect against potential threats, from pathogens such as bacteria and viruses. This is achieved through various layers of defense, beginning with physical barriers such as the skin and epithelial linings. Pathogens which breach these barriers meet the innate immune system, so called since it requires no previous exposure to mount a nonspecific response and induce an inflammatory state. Evasion of this innate immune response calls for further defense via the adaptive immune response. Initiated by the ongoing innate response, this adaptive response results in long-lasting immunity. These responses involve coordination between the circulatory system, lymphatic system, lymphoid organs and tissues, and specialized cells which move between these. In the context of transplantation, the consequence of this is detection and directed response against foreign cells. The immune system therefore must have the ability to identify “self” from “nonself” based on antigenic profile, in order to offer protection while preventing autoimmunity. From thorough investigation of skin grafts in humans, Medawar concluded graft rejection to be an immunological phenomenon (Gibson and Medawar 1943). Subsequent studies in rabbits further investigated this phenomenon through the comparison of autografts and homografts (allografts), confirming that the rejection of foreign skin occurs through “actively acquired immune reactions”

(Medawar 1944). The contribution of the different elements of the immune system to the transplant rejection response is discussed in the following sections. Since these responses will also be affected by the local resident cell population, and the nature of the transplanted tissues/cells themselves, the response to transplants is then discussed specifically in the context of the peripheral nervous system.

3.1 Innate Immunity

The innate immune response is the first mechanism of defense after injury. This involves a range of cell types such as mast cells, dendritic cells (DCs), basophils, eosinophils, natural killer (NK) cells, neutrophils, and macrophages (Morris et al. 2017). The main features of innate immunity are the ability to distinguish infectious nonself-molecules from self, and the ability to activate adaptive immune responses to those. Cells of the innate immune system are able to detect both structural pathogen-associated (PAMPs) and damage-associated molecular patterns (DAMPs) via pattern recognition receptors (PRRs), as termed by Janeway (1989, 2013). These consist of four families, Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors, C-type lectin receptors, and RIG-I-like receptors, and can react with broad groups of invasive pathogens. They are also triggered by various physical and metabolic insults, causing a response consisting of various soluble and cellular mediators of inflammation aiming to eliminate the threat (Farrar et al. 2013). TLRs, the most studied of the PRR families, possess fixed receptor structures, meaning they are capable of binding a limited ligand repertoire, and are highly conserved across evolutionary distant species (Brennan et al. 2010). Inflammatory cells of the innate immune system have limited clonal expansion and in general do not bring about immunological memory (Oberbarnscheidt et al. 2011). The innate immune system also includes noncellular mediators of microbial recognition such as complement proteins. The complement and TLR systems of innate immunity therefore demonstrate the integration of soluble and cellular components which provide immune surveillance of tissues (Farrar et al. 2013). As predicted by Janeway in 1989, mobilization of the innate immune system through PRR activation triggers both inflammation and the initiation of adaptive immunity, meaning it is not only responsible for first-line defense, but also shapes the adaptive immune response (Janeway 1989, 2013; Oberbarnscheidt et al. 2011).

Early on in the immune response to allografts, the nonspecific innate response predominates, followed by the donor-specific adaptive response. Procurement and transplantation of donor organs induces activation of the innate immune system resulting in tissue damage and the death of donor cells within the transplanted organ. This will contribute to innate immune activation such as graft inflammation, and result in tissue damage and the elimination of damaged cells within the graft (Ochando et al. 2019). Early inflammation independent of the adaptive immune response is considered to be an important factor in graft rejection (Moreau et al. 2013). Potential DAMPs such as reactive oxygen species and heat shock proteins can be generated by local tissue damage and reperfusion injury, as induced by the physical

process of isolation and manipulation of cells and organs for transplantation (Wood and Goto 2012). Necrotic cell death from reperfusion injury and surgical trauma has been shown to cause a strong inflammatory response, potentially due to mechanisms which have evolved to recognize cell death as an indicator of danger. Since necrotic cell death may be due to an infection, or occurring at a site where microbes may be introduced as a consequence of injury, these threats require rapid response. The instigation of an inflammatory response rapidly mobilizes defenses to mitigate this threat (Rock and Kono 2007). Hypoxia and reoxygenation of transplanted tissues causes rapid activation of the complement cascade, which can regulate B cells and antibody production, and could also contribute to the modulation of T cell responses (Zhou et al. 2007). During the early posttransplantation phase, innate immune system activation is a nonspecific response to tissue damage, irrespective of donor and host characteristics. The resulting release of proinflammatory cytokines which identify the transplant as a site of injury and inflammation triggers adaptive immunity, leading to the maturation of antigen-presenting cells (APCs), upregulation of costimulatory molecules, and further secretion of proinflammatory cytokines (Wood and Goto 2012; Moreau et al. 2013). The complement system, comprising soluble and cell surface proteins, also plays a role in the inflammatory response and host defense, as well as linking the innate and adaptive arms of the immune response (Zhou et al. 2007). Complement activation can occur through three different pathways leading to the clearance of immune complexes, invading pathogens, and injured cells. All three pathways result in the activation of the plasma protein C3 leading to inflammation, leukocyte recruitment, cytokine and chemokine release, oxygen radical production, and increased blood vessel permeability. There is evidence for a role of the complement cascade in complications during allograft transplantation, causing recruitment and activation of neutrophils and monocytes from the circulation into the allograft resulting in cell apoptosis and necrosis (Ochando et al. 2019).

3.2 Adaptive Immunity

The adaptive immune system includes T and B lymphocytes, which express highly specific antigen receptors which are diverse due to somatic gene rearrangement. For B lymphocytes, these surface receptors are antibodies (immunoglobulins), and for T lymphocytes they are T cell receptors (TCRs) which recognize nonself antigens. T cells expand clonally during antigen recognition, contributing to immunological memory (Yatim and Lakkis 2015). These antigen receptors allow recognition and targeting of nonself antigens which occurs through extensive lymphocyte proliferation and differentiation into specialized subsets. B lymphocytes become plasma cells which can produce antibody, and T lymphocytes into helper and effector/cytotoxic subsets which secrete a distinct set of cytokines. A distinct and specialized population of T cells are regulatory T cells (Tregs), characterized by expression of CD4, CD25, and FoxP3. These cells contribute to the suppression of T cell responses, in order to maintain self-tolerance within the organism (Sakaguchi 2000). Although, following a response, the majority of antigen-specific lymphocytes

ultimately die following this proliferation and differentiation, some memory lymphocytes remain to ensure that a second encounter with the same foreign antigen results in rapid targeting and elimination (Mueller et al. 2013).

3.3 The Major Histocompatibility Complex

The major histocompatibility complex (MHC) encodes the major antigens responsible for eliciting rejection, with transplantation of cells and tissues between MHC-identical individuals being readily accepted as opposed to between MHC-mismatched individuals, which are rejected in the absence of immunosuppression (Ayala-García et al. 2012). In humans, MHC antigens are collectively termed human leukocyte antigens (HLA) and are divided into three classes (I, II, and III) based on tissue distribution, structure, and function. The function of HLA class I and II surface molecules is to present peptides to T lymphocytes from either intracellular proteins, or proteins sampled from the extracellular environment, thus providing an extracellular representation of intracellular invasion (Germain 1994). Class III genes encode components of the complement system. In addition to playing a key role in antigen presentation, HLA molecules are recognition elements for immune cells surveying the body, allowing the determination of “self” and “nonself.” Immune cells will recognize specific peptides presented by APCs which bear identical MHC molecules to those expressed by the lymphocytes themselves, known as “MHC restriction” (Bolton and Bradley 2013). In the context of transplantation, immune cells respond to “nonself” MHC molecules with the initiation of a rejection response through antigen processing and presentation, described further below. To reduce the risk of rejection and the amount of immunosuppression administered, transplantation therefore requires close matching of donor and recipient HLA types. However, the large variability in potential HLA molecules expressed creates a challenge in matching between donor and host.

HLA class I and II have different functions reflected in their cellular distribution and have evolved to present cytoplasm-derived peptides or intracellular parasites (mainly viruses) via HLA class I, or bind peptides from extracellular proteins via class II. These are recognized by the two main T cell subsets, CD8⁺ (cytotoxic) and CD4⁺ (T helper) T cells, respectively (Germain 1994). All nucleated cells express varying levels of class I antigens, depending on the tissue, whereas class II antigens are normally expressed only by immune cells such as B cells, activated T cells, macrophages, DCs, and thymic epithelial cells (Klein and Sato 2000; Bolton and Bradley 2013). Importantly, upregulation of class II antigens on other cell types can be induced by the presence of proinflammatory cytokines such as interferon- γ (IFN- γ) (Klein and Sato 2000).

3.4 Antigen Processing and Presentation

The antigen-specific adaptive immune response follows later than the innate response via alloantigen presentation by APCs, allowing recognition by recipient T cells. All

cells which can express MHC Class II are able to present antigen to T cells; however, different cell types are able to process and present antigen at different efficiencies. Therefore, certain cells are considered to be “professional APCs,” including macrophages, DCs, and B lymphocytes (Schneider and Sercarz 1997; Trombetta and Mellman 2005). The so-called “immunological synapse” refers to these T cell-APC interactions (Norcross 1984) and can include any interface between lymphocytes or NK cells, and the cells which are being recognized (Huppa and Davis 2003).

In transplantation, due to the presence of both donor and host antigen presentation via MHC class I and II, the recognition of tissues can be mediated by “direct” and “indirect” antigen recognition. Direct recognition describes the recognition of intact MHC class II molecules on the surface of donor cells by host CD4 T cells. As these are recognized via MHC class II expression, this requires the presence of donor APCs. CD4 T cells will recognize and be tolerant to self HLA class II whereas the recognition of nonself antigen present on donor APCs, along with costimulatory molecule interaction, will activate the T cell. Indirect recognition allows host APCs, typically DCs, to internalize and process donor peptides and present their fragments for recognition by T cells and, therefore, does not require the presence of donor APCs (Gould and Auchincloss 1999; Siu et al. 2018). Activation and rapid expansion of T cells is initiated in response to the detection of alloantigen, requiring various related signals. The activation of naïve CD4 T cells has been suggested to be the most important event in initiating adaptive immunity, resulting in cytokine secretion, initiation of the adaptive immune response, and the induction of effector mechanisms leading to graft destruction.

Antigen processing and presentation requires the formation of MHC/antigen peptide complexes bound to the TCR to induce T cell activation, known as “signal 1.” This interaction determines which T cells will be activated, since each TCR will respond to a particular antigen (Janeway and Bottomly 1994). However, this signal alone is not sufficient for T cell activation; additional signals are required via interaction of costimulatory molecules on the T cell surface with their ligands on APCs, known as “signal 2” (Lafferty and Cunningham 1975). Stimulation of the TCR in the absence of costimulation will instead result in anergy, or apoptosis of the responding T cells (June et al. 1990; Schwartz 1990). There are a number of different costimulatory molecules which may support or inhibit T cell activation, classified into four distinct groups based on their structure, which are variably expressed on T cells (Kinnear et al. 2013). For example, the first pathway to be defined and most well characterized is the B7/CD28/CTLA-4 pathway. CD28 is constitutively expressed on around 80% of human naïve T cells (50% of CD8 and all CD4), with increased expression following T cell activation (June et al. 1990; Lenschow et al. 1996).

The CD28 ligands, the B7 molecules CD80 and CD86, are expressed on APCs and are upregulated on T cell activation (Greenwald et al. 2005). Following TCR stimulation (signal 1), costimulation via CD28/B7 ligands lowers the threshold for activation and increases the expression of IL-2 (signal 2), promoting growth and proliferation of T cells into effector T cells (Lenschow et al. 1996; Wood and Goto 2012). CTLA-4 is an additional B7 receptor, upregulated on T cells following activation and with a higher binding affinity for CD80/CD86. Following

upregulation on activated T cells, this creates competition for ligation with the B7 molecules, limiting CD28/B7 interaction and resulting in decreased IL-2 secretion, thus attenuating the T cell response (Walunas et al. 1994). Therefore, a balance between costimulation from CD28 and CTLA-4 is required for T cell activation and preventing continuation of this response (Alegre and Najafian 2006). After T cell activation, a number of factors can influence T cell differentiation via their effect on cytokine release from transplanted cells or tissues. These factors have been described in more detail above and include the immune status of the recipient, the degree of ischemia-reperfusion injury, donor-host mismatch, or immunosuppressive treatment used to prevent rejection (Wood and Goto 2012).

3.5 Transplant Rejection

Organ transplant rejection takes place through hyperacute, acute, and chronic rejection according to its time-course. Hyperacute rejection develops minutes to hours after the transplantation of vascularized grafts due to presensitization to donor tissue and is usually mediated by alloantibody and complement (Colvin and Smith 2005). This occurs due to the presence of antidonor antibodies in the recipient prior to transplantation. This type of rejection can be avoided by matching donor and recipient for blood type (ABO compatibility) and excluding the presence of anti-donor HLA antibodies in vitro (Moreau et al. 2013). Hyperacute rejection is also found in phylogenetically distant xenotransplantation, for example, primates (including humans) possess antibodies to the α -galactosyl epitope expressed by many porcine cell types, making immunosuppression essential for such xenografts (Sandrin et al. 1993). More recently, the genetic modification of pigs has aimed to develop a potential xenogeneic source of donor tissue for transplantation by deleting xenoantigens to which humans have preformed antibodies, and by transgenic expression of protective proteins such as human complement proteins (Cooper et al. 2021). Solid organ xenotransplants from Gal α 1-3Gal β 1-4GlcNAc (Gal) knockout pigs and baboons require either additional genetic manipulation, or immunosuppressive treatments, to promote survival (Zhong 2007). Long-term survival of genetically multimodified α 1,3-galactosyltransferase knockout pig hearts expressing human CD46 (complement) and thrombomodulin pig hearts following transplantation into baboons has been demonstrated recently; however, recipient animals also required extensive immunosuppressive treatment (Längin et al. 2018). These requirements for further treatments to promote the survival of organ xenografts mean, as yet, genetically modified porcine donor tissue is unlikely to be a suitable source of tissue for peripheral nerve transplantation. As well as preformed natural antibodies, potential transplant recipients may possess antibodies developed due to prior exposure to foreign antigens (Auchincloss and Sachs 1998). Potential prior antigen exposure, for example, through pregnancy or previous transplant, is therefore an important consideration in the selection of transplant recipients. Acute rejection occurs from 1 week to several months after transplantation and is thought to result from two immunological mechanisms, either alone or in combination. These are alloimmunity, following the development of

antigen-specific T cells for donor MHC, and a B-cell-dependent process resulting in acute humoral rejection through the development of donor-specific antibodies (Colvin and Smith 2005; Moreau et al. 2013).

Chronic rejection, now the leading cause of rejection of solid organ transplants, develops over months to years and can also be mediated by humoral or cellular mechanisms (Moreau et al. 2013). Circulating HLA-specific antibodies are often found in patients with long-term organ allografts, and the presence of these antibodies greatly increases the risk of chronic rejection (Colvin and Smith 2005). If the recipient has not previously been exposed to the transplant alloantigen, B cells do not directly participate in the acute rejection response. However, after sensitization from, for example, a previous transplant or blood transfusion, antibodies will be produced which can cause hyperacute and accelerated rejection. This may also contribute to chronic rejection of transplants, due to the development of alloantibody (Sayegh and Turka 1998). Alloantibodies target foreign MHC molecules, but antibodies specific for minor histocompatibility antigens, endothelial cells, and blood group antigens can also contribute to rejection. Although the main alloantigens responsible for inducing transplant rejection are MHC molecules, host or recipient MHC can also present minor histocompatibility antigens and result in rejection even in MHC-matched transplants (Peugh et al. 1986).

4 Peripheral Nervous System Immunology

As for the central nervous system (CNS), the peripheral nervous system (PNS) is considered a site of relative “immunological privilege.” These areas are separated from the periphery by barriers which can limit the passage of immune cells, and lack a resident population of immune cells themselves; however, this privilege is not absolute. The blood-nerve-barrier (BNB) in endoneurial blood vessels prevents the movement of substances including circulating immune cells via tight junctions. The perineurium, composed of layers of perineurial cells connected by tight junctions, offers the main barrier between the endoneurium and extrafascicular tissues (Peltonen et al. 2013). Tight junctions comprise a complex network of transmembrane and peripheral proteins to restrict the flow of ions and molecules into the endoneurium, including claudins, occludins, junctional adhesion molecules, and zonula occludens complexes (Richner et al. 2019). The BNB and perineurial barriers act as diffusion barriers preventing access to the endoneurial compartment; however, this restriction is not complete. Soluble factors and cellular elements can enter the PNS in areas where the barrier does not exist, including root entry and exit zones where CNS and PNS segments meet, and nerve terminals (Kieseier et al. 2006). In addition, a lack of lymphatic drainage and limited passage of lymphocytes separates the endoneurial environment, reducing foreign antigen presentation in the peripheral lymphoid organs. Similar to the CNS, along with these physical barriers, cells in the nerve parenchyma generally lack constitutive MHC expression and are not professional APCs (Hughes 1992). Professional APCs are required to process and present

antigen to T cells via MHC molecules in order to initiate an immune response (Wekerle et al. 1987).

However, as for the CNS, immunological privilege in the PNS is no longer considered to be absolute. Constant immunological surveillance by patrolling T and B lymphocytes is operative, and following activation, for example, in a disease state, these cells can cross the BNB and increase permeability of the barrier thus also allowing the access of antibodies irrespective of the specificity of antibodies or T cells (Pollard et al. 1995; Spies et al. 1995). This disruption has been shown to include loss of tight junctions and separation of endothelial cells (Powell et al. 1991). The BNB is also compromised after PNI, often via a breach at the injury site, and additionally following axonal degeneration, the barrier is compromised along the length of the nerve distal to the injury up to at least 4 weeks postinjury (Gray et al. 2007). The peak inflammatory response occurs after 4–7 days, and this corresponds with maximal perineurial permeability (Weerasuriya and Hockman 1992). Thus, increased permeability is also induced following the administration of pro-inflammatory cytokines such as TNF- α (Spies et al. 1995). Increased permeability under these circumstances will allow cells and blood-borne factors to enter the nerve and facilitate tissue repair. In the steady state, local resident macrophages make up around 2–9% of the endoneurial population in peripheral nerves (Monaco et al. 1992; Mueller et al. 2003). Resident macrophages express MHC molecules and complement receptors, allowing them to perform antigen presentation and surveillance.

Although transplanted nerve grafts do not contain MHC class II expressing professional APCs, the secretion of inflammatory cytokines will cause its upregulation on donor cells within the transplanted tissue thus permitting direct presentation to host lymphocytes. Endoneurial macrophages show up-regulation of MHC class II following the secretion of IFN- γ (Hughes 1992). Similarly, SCs constitutively express low levels of MHC class I but no MHC class II molecules. However, they have the capacity to upregulate MHC class I and express class II following stimulation by IFN- γ and tumor necrosis factor α (TNF α), or in coculture with activated T cells. Consequently, upregulation is also observed in vivo in an inflammatory environment due to exposure to these cytokines (Hartlehnert et al. 2017). Additionally, molecules such as ICAM-1 which can exert costimulatory functions are also upregulated by cytokines (Gold et al. 2006). Thus, SCs have been identified as inducible APCs, capable of presenting antigen to T cells, including presenting antigenic epitopes of myelin components following myelin phagocytosis by SCs (Wekerle et al. 1986). In addition, SCs are a source of proinflammatory cytokines including interleukin-1 (IL-1), IFN- γ , and TNF α , showing a role in the modulation of local immune reactions in the PNS. They are also able to modulate local immune reactions in the PNS via the release of humoral factors which can inhibit or stimulate T cells (Gold et al. 2006).

4.1 The Immune Cell Response to Nerve Injury

The innate immune system plays an important role in recovery from PNI. The injury site is invaded by various immune cells, with phagocytic neutrophils and monocyte-

derived macrophages arriving quickly following injury (hours to days), and lymphocytes accumulating in the distal stump after a week or so (Gaudet et al. 2011). Regeneration is dependent on Wallerian degeneration in nerve segments distal to the lesion site, through which severed axons grow back to target tissues. Axons regenerate readily after crush but not transection, resulting in variation between the cellular and molecular events following a crush injury or a cut. Wallerian degeneration is characterized by the breakdown of axons, recruitment and activation of macrophages, to remove degenerated axons and myelin along with resident SCs and resident endoneurial macrophages (Mueller et al. 2003; Rotshenker 2011). As described previously, cytotoxicity and cell death pathways are important components of the response to infection, disease, or injury. In the context of nerve injury and pain, inflammation and cytotoxicity are key mechanisms of the cellular immune response, designed to facilitate the repair of injured tissue (Davies et al. 2020). Following trauma, local cytokine and chemokine release by immune and non-immune cells (e.g., fibroblasts) leads to the accumulation of bone-marrow-derived macrophages, which are not normally present in the intact PNS (Shamash et al. 2002). The first phase is characterized by proinflammatory cytokine (e.g., TNF α ,) production, and the second phase by anti-inflammatory cytokines (such as IL-10) and macrophages of the M2 phenotype, involved in tissue repair (Rotshenker 2011). Macrophages, along with SCs, are important for the removal and degradation of myelin debris during Wallerian degeneration and exert cytotoxicity via phagocytosis (Stoll et al. 1989). Degeneration of the peripheral nervous system requires this immune response and predicates successful regeneration. This debris clearance is also facilitated by complement, with components synthesized by SCs to attract macrophages to the injury site, and a reduction in autoimmunity through the clearance of apoptotic cells (Cashman and Hoke 2017). The interaction between different arms of the immune response (complement, innate, adaptive) and the cell types concerned is complex, and the exact roles of each in debris clearance are still under investigation. However, it has been shown that deficiencies in adaptive immunity, as tested in immunodeficient mice, do not prevent degeneration or debris clearance, showing that the innate immune response is dominant in the clearance process early after injury (Cashman and Hoke 2017).

4.2 The Immune Response to Peripheral Nerve Grafts

The presence of therapeutic cells within nerve grafts greatly improves the outcomes of nerve repair; however, if donor cells elicit a rejection response from the host immune system, this can have a negative effect on regeneration (Gulati and Cole 1994). Although clinical studies can provide information about the efficacy of peripheral nerve autografts and allografts, preclinical studies include grafts of varying genetic disparity. The peripheral nerve autograft is commonly used as well as “isografts” between different donor animals from within an inbred strain, which are assumed to not differ genetically. These can be compared against mismatched allografts between rats of differing inbred strains. In the literature, outbred rat stocks are often frequently used, which will increase variability in transplant studies

between even littermates due to potentially inconsistent differences between animals. While no immune response is elicited to peripheral nerve autografts/isografts, allografts induce a response initiated by the recognition of foreign histocompatibility antigens on donor tissue and result in destruction of the graft via the mechanisms described above (Gulati 1998). Transplanted nerves are, however, classified as tissues of low immunogenicity, as the immune response to allografts has been reported to be lower than in other tissues such as muscle and skin (Hettiaratchy et al. 2004; Siemionow and Sonmez 2007).

As previously described, transplanted nerve tissue contains a range of different cell types, which may or may not be recognized by the host immune system. The cellular components of the endoneurium have been concluded to be the immunogenic component of the graft. Among these, SCs have been suggested to have the highest antigenicity, due to the upregulation of MHC Class II following transplantation and their subsequent role as APCs (Wekerle et al. 1986; Hughes 1992; Siemionow and Sonmez 2007). As decellularization of nerve allografts reduced the immune response to transplanted peripheral nerve, living donor cells within the implanted nerve must be capable of inducing an immune response (Gulati and Cole 1994). After transplantation, the BNB is broken down, and rapid revascularization of the allograft occurs from the proximal and distal segments of the host nerve via direct anastomosis between the host and graft vessels or neovascularization. Revascularization allows progressive invasion of blood-borne macrophages and lymphocytes into the donor nerve, initiating local inflammatory responses and allowing recognition of donor-specific antigens (Gulati 1998; Siemionow and Sonmez 2007). Established long-term rat allografts in immunocompromised or immunosuppressed hosts have been shown to be protected by normal permeability barriers, which were assumed to be from donor perineurium and endoneurial vasculature (Zalewski et al. 1993). However, lymphocyte infiltration into nerve allografts through compromised barriers will occur in immunocompetent hosts (Gulati 1998; Hellenbrand et al. 2016).

Living SCs within peripheral nerve grafts can act as APCs and have been considered the main target in nerve allograft rejection, though the relative contribution of the direct and indirect pathways is not clear. Ray et al. (2011) identified a more significant contribution of the indirect pathway in allorecognition than previously assumed, via allotransplantation of MHC—/— mouse sciatic nerve grafts into wild type hosts and vice versa, thus isolating the indirect and direct pathways (Ray et al. 2011). The authors found that the elimination of the indirect pathways reduced the host response, improving regeneration compared to allografts. However, eliminating only the direct pathways yielded no improvement in comparison to allografts. This seems to suggest a predominance of the indirect pathway, though this may be due to the relative lack of professional APCs in nerve allografts. Since SCs are facultative APCs, indirect presentation of antigen may contribute to a larger portion of the host response compared to grafts containing, for example, professional APCs such as DCs (Ray et al. 2011). Although different cell types, such as SCs, can present antigen, it is known that the efficiency of antigen presentation can be very different. Thus, some cell types are considered professional APCs, including B lymphocytes,

macrophages, and DCs. Of these, B cells and DCs are effective APCs which function for antibody secretion and the initiation of T cell responses, respectively. Although, in addition to MHC-I, macrophages express MHC-II and costimulatory molecules, these are at much lower levels, and therefore macrophages are less efficient at antigen presentation than B cells or DCs (Trombetta and Mellman 2005).

Transplantation of nerve isografts has been shown to produce superior regeneration to nerve allografts (Gulati and Cole 1994; Kvist et al. 2007). Early research sought to quantify the immune response to nerve allografts and identify the associated immunological mechanisms using rat allograft models with mismatching RT1 (rat MHC) histocompatibility (Mackinnon et al. 1982). Nerve allografts were found to be less immunogenic than skin allografts with only minor differences in donor/host histocompatibility, which may explain the success of some clinical nerve allograft cases. The response was shown to be composed of dense infiltration with activated lymphocytes and macrophages first in the epineurial tissues, while the perineurium conferred a temporary barrier to these cells before their infiltration. Such infiltration was not observed in isografts, or in the intact nerve (Mackinnon et al. 1982). When matching for major or minor histocompatibility antigens in nonimmunosuppressed rats, it was found that host nerve fibers regenerated functionally through 2 cm but not 4 cm nerve allografts, irrelevant of the level of matching (Zalewski and Silvers 1980). This suggests a sufficient immune response to prevent functional regeneration through a longer graft, even from minor antigens.

The time line for the rejection response to peripheral nerve allografts varies between reports. Some show an acute rejection response, peaking around 7–10 days posttransplantation, with full clearance of donor cells by 21 days (Ansselin and Pollard 1990). Others find an ongoing increase in infiltration of immune cells within the donor nerve up to at least 4 weeks posttransplantation (Gulati 1998). When investigating the contribution of different arms of the immune response to peripheral nerve allografts in mice, Ishida et al. found a peak in cytotoxicity due to cytotoxic T cells at around 11 days posttransplantation. Specific antibody production peaked in serum at 3–4 weeks after grafting (Ishida et al. 1990). These data suggest the cellular immune response is involved in the early stages of rejection, with the humoral response due to the development of specific serum antibodies developing later. Strong activity to both MHC class I and class II antigens was also reported in the study, suggesting expression of both was present in the grafted peripheral nerve tissues. Antibody was detected for up to 3 months posttransplantation, suggesting antibody-induced cytotoxicity to host antigen can remain active for long periods in vivo (Ishida et al. 1990).

Lassner et al. studied the cellular mechanisms of rejection in rat-peripheral nerve allografts, finding highly elevated donor-specific MHC class I and II expression 1 week after transplantation, with specific expression on donor myelin and interstitial cells close to the coaptation sites. After 2 weeks, this had largely reduced and was no longer detectable by 6 weeks. Finally, after 12 weeks, signs of regeneration were evident. The authors suggest that nerve allograft rejection is directed at vascular endothelium and myelin as a product of SCs, and that donor-derived SCs are no longer present in rejected allografts at 6 weeks. Subsequently, the graft acts as an

acellular allograft, with intact tissue architecture, permitting some regeneration (Lassner et al. 1989). More recently, Roballo and colleagues sought to evaluate the immune response to 1 cm nerve autografts and allografts in the sciatic nerve of immunocompetent rats, by characterizing cellular processes at various timepoints posttransplantation. The authors reported a significant elevation in immune cell density within autografts 3 days after transplantation compared to allografts, in particular of macrophages, NK cells, and cytotoxic (CD8⁺ T cells) (Roballo and Bushman 2019). This subsequently reduced up to 28 days posttransplantation, except for the regulatory T cell (Treg) marker CD25, which spiked at day 7. In allografts, no consistent elevation of effector T cell density was found compared to autograft. This is suggested to be due to the immunological privilege of the peripheral nerve. It is unclear why this would be the case given the ability of SCs within the graft to express MHC-II and act as APCs to present antigen to T cells accumulating within the graft. In addition, no increase in CD25⁺ Tregs was found in autografts; therefore, the lack of rejection could not be mediated by the repression of effector T cells by Tregs (Roballo and Bushman 2019). Indeed, it has been shown that peripheral nerve cells and tissues display several immunological hallmarks, all nucleated cells would express MHC Class I within the peripheral nerve, and resident macrophages and DCs would be capable of presenting antigen via MHC Class I and II. Mouse-peripheral nerve cells have been shown to be robustly immunogenic in vitro (Ishida et al. 1990). A confounding factor in this experiment is the use of donor tissue from an outbred rat strain, which may increase variability in the data due to chance potential tissue compatibility between donor and host (Evans et al. 1994).

The immune response to peripheral nerve allografts also poses a challenge for the development of novel tissue engineering solutions for peripheral nerve repair. Tissue-engineered constructs with a cellular component have been shown to produce greater functional outcomes than nerve conduits alone (Carvalho et al. 2019), though this has implications for the immunogenicity of the graft. Although it is possible that autologous cells could be used to develop grafts, a more cost-effective solution for an “off the shelf” living nerve construct would be using allogeneic cells. The potential for the generation of cell banks of different haplotypes to attempt to HLA match transplants to individuals has been proposed (Taylor et al. 2012), though it has been shown that HLA matching only may be insufficient without the use of further immunosuppression. Even delivering matched transplants to the brain, another “immunologically privileged site” does not afford sufficient protection from the immune response (Aron Badin et al. 2019). Reports have suggested that only very low levels of MHC class I and II are expressed on immature cells (Odeberg et al. 2005) and on embryonic stem cells (ESCs) (Drukker et al. 2002), and therefore that these donor cells may be less susceptible to rejection on transplantation. However, subsequent differentiation of cells in culture was shown to cause upregulation of MHC class I expression, and the addition of IFN- γ also induced MHC class II upregulation suggesting these cells are likely still immunogenic on transplantation (McLaren et al. 2001; Odeberg et al. 2005; Drukker et al. 2006). Variable immunogenicity has also been demonstrated in induced pluripotent stem cells (iPSCs), with autologous iPSC transplant rejection observed in mouse hosts

(Zhao et al. 2011). Following the rejection of cellular components of nerve allografts, tissue architecture remains intact, allowing function as a nerve conduit. However, the regenerative potential of such a conduit is reduced compared to a graft containing viable therapeutic cells such as SCs (Pan et al. 2019). These findings indicate that transplantation of cellular nerve grafts is likely to require host immunosuppression, or an alternative approach, to prevent the rejection of grafts and allow regeneration.

An additional consideration for the use of artificial nerve guidance conduits is the response to the biomaterials themselves. The use of natural materials such as decellularized tissues and tissue-derived hydrogels may elicit a foreign body response and adaptive immune response due to the presence of native molecules resident in the tissue (Morris et al. 2017). The foreign body response is initiated by implantation of material as a response to the injury, following a similar but altered response to normal wound healing and repair mechanisms. This begins with protein adsorption to the material surface, where a provisional matrix forms at the tissue/material interface comprising a thrombus/blood clot (Anderson et al. 2008). This provisional matrix contains chemoattractants, cytokines, and growth factors which induce proliferation and activation of macrophages and other cell populations involved in the inflammatory response. Acutely, this inflammatory response is characterized by the invasion of neutrophils and mast cells which interact with surface-adsorbed proteins and release inflammatory cytokines. Depending on the extent of injury at the implant site, this acute inflammatory response usually resolves in less than a week. Recruited macrophages infiltrate and become the predominant cell type in the peri-implant space. Ultimately, these begin to fuse and become multinucleated foreign body giant cells, which further release cytokines and growth factors to promote the invasion of fibroblasts and induce fibrotic deposition of ECM, indicating a chronic inflammatory response. Resolution of the acute and chronic response by around 2 weeks can occur with biocompatible materials. By around 4 weeks, this response results in the encapsulation of implants in a largely avascular collagenous capsule (Morris et al. 2017).

Although the focus of this chapter is on the host response to allografts or transplants containing allogeneic cells, with the use of biomaterials in tissue-engineered constructs or for growth factor/drug delivery, it is important to consider that the innate response to biomaterials can have an influence on the adaptive response to associated antigens (Sefton et al. 2008). Biomaterials may be used as vehicles for the delivery of cells to the peripheral nervous system, and therefore may potentiate the immune response toward the cells via the adjuvant effect of the biomaterial (Babensee 2008). The main mechanism for this is through the maturation of APCs, specifically DCs. DCs have been shown to infiltrate and interact with biomaterials and to mature in their presence, increasing the expression of costimulatory and MHC Class II molecules and secretion of proinflammatory cytokines and inducing T cell proliferation *in vitro* (Yoshida and Babensee 2004). This effect has been shown to be dependent on contact with biomaterial, and maturation of DCs differs depending on the type of biomaterial (Babensee 2008). The recognition of biomaterials by DCs is thought to occur via the same mechanisms as the response to

pathogens, through PRRs which may recognize molecular patterns associated with biomaterials, similar to PAMPs. In addition, the protein layer adsorbed to the biomaterial surface may provide ligands which serve as “danger signals” or DAMPs. These can prime the system for an enhanced immune response which will be targeted at the antigenic component of the transplant, the donor cells. Thus, the avoidance of these signals through minimizing tissue injury during implantation and optimal biomaterial selection should reduce DC maturation and the resulting adaptive immune response to transplanted cells.

5 Strategies for Preventing Rejection of Peripheral Nerve Transplants

A number of strategies have been investigated for preventing allograft rejection. Preventing the immune response to transplants could be achieved through inhibiting the host immune response, or modification to lower the immunogenicity of the transplanted tissue itself (Ishida et al. 1990). A simple approach to reducing the immunogenicity of nerve allografts has been achieved through decellularization, removing the cellular component which is responsible for inducing the immune response to grafts, and leaving the nerve bridge structure intact.

5.1 Pharmacological Immunosuppression

In organ transplantation, the introduction of cyclosporine A (CsA) yielded great improvements in the preservation of graft function as well as prolonging patient survival. Transplantation became a possibility for not only acutely life-threatening conditions, but also those which adversely affected quality of life and longevity (Brenner et al. 2002). To prevent the rejection of nerve transplants through suppression of the immune response, chronic systemic administration of immunosuppressant drugs is required, associated with secondary risks of toxic effects including nephrotoxicity and hepatotoxicity (Rezzani 2006; Teh et al. 2011). Treatment also requires long-term treatment at high doses to achieve efficacy (Rustemeyer et al. 2010). The aim of peripheral nerve transplantation is to restore function and thus improve quality of life. Since this is not treating a life-threatening condition, the risks associated with systemic immunosuppression may not be warranted (Mackinnon et al. 1992). Benefits of allografting and the potential outcomes must therefore be carefully weighed against the risks associated with immunosuppression. However, unlike other transplants, the function of a nerve graft is to provide a conduit to facilitate axonal regeneration across a nerve gap. Once host axons have traversed the nerve conduit and achieved functional connections with target end organs, living donor cells within the graft may be replaced with host cells including vasculature and SCs (Mackinnon et al. 1992). This scenario gives the possibility of temporary immunosuppressive treatments for peripheral nerve allografts or engineered nerve grafts containing allogeneic cells (Midha et al. 1993).

Cyclosporine A (CsA) is an immunophilin ligand which binds cyclophilin, blocking the phosphatase activity of calcineurin which is essential in T cell activation and therefore prevents immune response initiation (Ho et al. 1996). CsA or other immunosuppressant drugs can be administered individually, or in combination to promote transplant survival. Effective regeneration across peripheral nerve allografts following immunosuppression with CsA and FK506, an alternative immunophilin ligand with higher potency, has been demonstrated in rodents. Successful immunosuppression resulted in comparable regeneration in allografts as compared to autografts/isografts (Bain et al. 1988; Udina et al. 2003). Treatment with FK506 has also been shown to be effective in rescuing rejecting rat peripheral nerve grafts, albeit within a specific window of 10–14 days posttransplantation (Feng et al. 2001). In addition to its immunosuppressive action, FK506 has been shown to accelerate regeneration both in vitro and in vivo in rodent models of peripheral nerve injury even after short-term administration (Gold et al. 1994, 1995; Rustemeyer et al. 2010; Yan et al. 2012), discussed in chapter ► [“Drug Therapies for Peripheral Nerve Injuries.”](#) However, due to chronic side effects associated with systemic delivery of FK506 and global immunosuppression, this is not in regular clinical use for the treatment of PNI.

During regeneration across a nerve allograft, donor-antigenic components within the graft such as SCs are gradually lost and replaced by host components, suggesting only temporary immunosuppression may be required to achieve successful regeneration across a nerve allograft (Midha et al. 1993, 1994). Temporary immunosuppressive treatment, with CsA in rats for 8 weeks after nerve allografting, resulted in graft rejection at 14 weeks associated with a decrease in function. However, long-term assessment (14–20 weeks) showed improvement in motor function and morphological parameters, demonstrating that although initial graft rejection resulted in short-term functional decline, good long-term functional regeneration was achieved (Mackinnon et al. 1992). Clinically, immunosuppressive treatment with CsA or FK506 of individuals receiving peripheral nerve allografts has also been shown to sustain nerve function after discontinuation of treatment (Mackinnon et al. 2001). Additionally, local delivery of immunosuppression is a potential option which could allow the survival of allogeneic cells for peripheral nerve repair, while avoiding associated side effects. Developments in the field of materials science have yielded various drug delivery devices which can provide sustained, targeted, and controlled release of drugs to specific tissues while reducing systemic toxicity. For example, controlled release formulations have been developed for delivery of calcineurin inhibitors Cyclosporine A (CsA) and FK506, in the form of micro-/nanoparticles or hydrogels, which have shown suppression of T cell proliferation in vitro and in vivo in various rodent allograft models (reviewed in (Fisher et al. 2015)). Recently, improved regeneration through fresh rat sciatic nerve allografts was shown following the application of PLGA FK506 microspheres suspended in a fibrin hydrogel over the superficial surface of the length of the nerve graft (Zuo et al. 2021), and some evidence of efficacy in vivo (Fisher et al. 2015; Dzhonova et al. 2018).

5.2 Costimulation Blockade

As previously described, two signals are required to initiate an immune response, with the formation of the MHC-T cell receptor complex and the interaction of costimulatory molecules with their ligands. This suggests that blocking these costimulatory signals during TCR stimulation could prevent the T cell response, allowing long-term allograft survival (Sayegh and Turka 1998). Therefore, a body of research aims to manipulate this interaction via costimulatory blocking to produce tolerance to transplants and avoid adverse effects of immunosuppressant drugs. Blocking costimulatory signals is an approach to manipulate the immune system to promote allograft survival which has been investigated previously in organ transplantation. A number of experiments have reported successful tolerance to transplants following costimulatory pathway blocking. A dual treatment with CD40L antibody following an injection of allogeneic donor splenocytes into mouse hosts was shown to promote skin graft survival for at least 100 days without further immunosuppression, though alloresponsiveness was reported to have increased at this point (Markees et al. 1997). As no chimerism was reported, the authors proposed a state of “split tolerance” had been achieved. In the context of nerve transplants, even temporary accommodation of a graft would be acceptable to allow survival until regeneration across a nerve gap could be achieved. Further successful costimulatory blocking has shown that administration of CTLA4-Ig, which blocks the CD28-mediated costimulatory signal, inhibits the immune response to vascularized cardiac allografts in mice for over 100 days. In contrast to the response described by Markees et al., the authors reported donor-specific transplant tolerance when tested with donor-specific or third-party skin grafts (Pearson et al. 1994). Pearl et al. successfully prevented the rejection of xenogeneic human ESCs and iPSCs after intramuscular injection in adult murine hosts using a combination of three costimulatory receptor-blocking antibodies (CTLA4-Ig, anti-LFA-1, and anti-CD40L). Outcomes were comparable to those treated with immunosuppressant drugs, and mice were found to be tolerant to donor cells, showing specific T cell anergy with no detrimental effects on the hosts’ immunity to other cell types (Pearl et al. 2011).

Costimulatory blockade via the same triple treatment (CTLA4-Ig, anti-LFA-1, and anti-CD40L) successfully suppressed the immune response to mouse nerve allografts, though with no effect on short-term nerve regeneration (up to 9 days), with better regeneration still observed in animals receiving nerve isografts (Kvist et al. 2007). Subsequent investigation of the effects on long-term regeneration (49 days) confirmed continued suppression of the immune response and, although no difference in regeneration was observed compared to isografts again, the authors reported increased myelination in treated animals (Kvist et al. 2008). Additional research has found suppression of the immune response and improvements in regeneration of mouse allografts using anti-CD40L antibody treatment to block costimulatory binding, which was validated in two different mismatched allograft models, though again outcomes did not exceed that of the nerve isograft (Jensen et al. 2004; Brenner et al. 2004). As reported previously with anti-CD40L treatment

(Markees et al. 1997), tolerance to allografts was not conferred, and subsequent immunological challenge with a repeated allograft resulted in a strong immunological response (Brenner et al. 2004). Further investigation tested treatment with increasing degrees of costimulatory blockade, CD40/CD40L or CD28 pathway blocking alone (anti-CD40L antibody or CTLA4-Ig), double treatment with both anti-CD40L and CTLA4-Ig, or triple treatment with the addition of ICOS/ICOSL pathway blocking agent anti-ICOSL (Tai et al. 2010). Increasing degrees of costimulatory blockade were shown to reduce the host response to donor antigen with a clear effect on regeneration showing that triple treatment could improve regeneration comparable to isografts. This depended on the addition of ICOS pathway blocking, suggesting optimal nerve allograft function requires a higher immunosuppressive requirement than allograft survival alone. Blocking this pathway has a specific effect on activated T cells, which may achieve this increased immunosuppressive requirement (Tai et al. 2010).

Clinically, costimulatory blocking agents are used, though in combination with pharmacological immunosuppressive treatments to reduce repeated dosing and risks of nonspecific immunosuppression (Tai et al. 2010). Data suggest that blocking multiple pathways for costimulation may be a promising approach for peripheral nerve allografting. It also is an appropriate method of temporary immunosuppression well suited to the requirements for nerve allografts, allowing prolonged unresponsiveness to transplanted tissues or cells while regeneration and repopulation of the graft with host cells occurs.

5.3 Cotransplantation of Regulatory T Cells

Under certain conditions, activation of CD4⁺ T cells can secrete cytokines which may not be associated with a rejection response. For example, CD4⁺CD25⁺FoxP3⁺ Tregs play a role in maintaining tolerance to self-proteins and avoiding the development of autoimmunity and can secrete the anti-inflammatory cytokine IL-10. Therefore, it is hypothesized that balancing Tregs over effector cells could determine transplantation tolerance in the host. This could be achieved either through the *in vivo* induction and expansion of Tregs, or the infusion of *ex vivo* expanded cells (Safinia et al. 2015). It has been shown that Tregs are required for the induction of tolerance to allogeneic antigens, including through costimulatory blocking (Taylor et al. 2001). The use of Tregs to induce tolerance to transplants allows the possibility of genetic reprogramming of Tregs to tune subpopulations, or expand alloantigen-specific cells (Singer et al. 2014; Safinia et al. 2015). The establishment of long-term tolerance requires adoptively transferred Tregs to survive and expand within the recipient, or for the a tolerogenic phenotype to be induced on other T cells within the host.

Transplantation of allogeneic Tregs along with peripheral nerve allografts in rats has been attempted as a method of local, temporary immunosuppression, by delivery around grafts via a degradable hydrogel (Santos Roballo et al. 2019). The authors aimed to ensure sufficient numbers of Tregs at the graft site through incorporation

into a hydrogel which could be administered across the whole site of the nerve transplant and polymerized in situ with UV light. It was shown in vitro that 84% of Tregs encapsulated in hydrogels escaped over 14 days and remained viable, and this was hypothesized to be sufficient to provide immunomodulation during the period of infiltration of nerve grafts with immune cells. After 21 days in vivo, data suggested that implanted Tregs had infiltrated and integrated within the transplanted nerves, and a reduction in infiltration of host CD4⁺ T cells was also reported. After 20 weeks, improved regeneration was reported in allografts delivered with Treg hydrogels compared to vehicle or untreated allografts, measured with compound muscle action potential recordings and quantification of axons (Santos Roballo et al. 2019).

5.4 Modifying the Immunogenicity of Donor Cells

Using genetic manipulation to avoid the rejection of transplanted cells is a potential approach to generating a “universal donor” cell. MHC expression is the largest contributor to the rejection response, with higher levels of MHC Class I expression relating to increased transplant rejection and lower with transplant survival (Mason et al. 1986). Therefore, deletion of both classes has been attempted as a method of evading immunological detection of transplanted cells (Bradley et al. 2002). Low levels of MHC Class I and II expression in undifferentiated human ESCs have been reported, though subsequent differentiation is found to elevate MHC-I expression. The upregulation of both MHC-I and II is also observed following the addition of IFN- γ (Drukker et al. 2002). Therefore, although lower MHC expression in undifferentiated ESCs may indicate lower immunogenicity, following differentiation (likely to occur prior to transplantation to avoid tumorigenicity) and upon transplantation and induction of the inflammatory response and associated cytokine release, MHC will be upregulated and increase the chance of recognition and elimination by the host immune system. However, the level of MHC expression is not the only factor relating to rejection, and a lack of MHC expression on allogeneic cells has been shown to activate NK cells which subsequently target transplanted cells for rejection (Phillips et al. 2013). There is currently little information on the potential contribution of minor histocompatibility antigens in the rejection of hESC-derived cells, though it is accepted that donor cells should be as genetically similar as possible to the recipient. Male hESC lines should not be used as donor cells for transplantation into females, as proteins encoded by the Y chromosome are not present in the host (Roopenian et al. 2002).

Advances in cell engineering and gene delivery offer the potential for genetic modification of graft cells to reduce the potential for immunological rejection following transplantation, as opposed to manipulating the host through immunosuppression. Engineering cells to reduce or eliminate HLA expression could provide a method to achieve this and offer a “universal” cell line for transplantation. Silencing or deletion of essential molecules for HLA expression, discussed in Cicciarelli et al., can be achieved through various methods (Cicciarelli et al. 2013). Donor cells can

also be modified by the inhibition of costimulatory pathways, and ectopic expression of immunosuppressive molecules. However, as yet, immune tolerance of “universally compatible” donor cells has not been demonstrated in humans, and these cells are also potentially still susceptible to some types of immune responses, including the NK cell response described previously (Zheng et al. 2016).

6 Conclusions

The development of novel approaches to surgical nerve repair requires a consideration of the host immune response to transplants, as well as the development of the cellular and material components of the nerve transplant. Important consideration should be taken to the inflammatory response elicited by transplantation, the potential foreign body response associated with the use of tissue-engineered constructs, and the immunological response to donor cells within the grafts. It is essential to consider the choice of biomaterial and associated response, and achieve noninvasive delivery of tissue-engineered constructs to reduce tissue damage and avoid increasing the adaptive response to implanted cells. Developing strategies to overcome immunological rejection of allografts, or to modify transplants to reduce immunogenicity, could provide a sustainable source of donor nerve for transplantation. This would alleviate the issues associated with autografting, and improve functional outcome by providing a supply of sufficient donor nerve of the relevant size for repair.

References

- Alegre M-L, Najafian N (2006) Costimulatory molecules as targets for the induction of transplantation tolerance. *Curr Mol Med* 6:843–857
- Anderson JM, Rodriguez A, Chang DT (2008) Foreign body reaction to biomaterials. *Semin Immunol* 20:86–100
- Ansselin AD, Pollard JD (1990) Immunopathological factors in peripheral nerve allograft rejection: quantification of lymphocyte invasion and major histocompatibility complex expression. *J Neurol Sci* 96:75–88. [https://doi.org/10.1016/0022-510x\(90\)90058-u](https://doi.org/10.1016/0022-510x(90)90058-u)
- Aron Badin R, Bugi A, Williams S et al (2019) MHC matching fails to prevent long-term rejection of iPSC-derived neurons in non-human primates. *Nat Commun* 10:4357. <https://doi.org/10.1038/s41467-019-12324-0>
- Auchincloss H, Sachs DH (1998) Xenogeneic transplantation. *Annu Rev Immunol* 16:433–470. <https://doi.org/10.1146/annurev.immunol.16.1.433>
- Ayala-García MA, Yebra BG, Liliana A et al (2012) The major histocompatibility complex in transplantation. *J Transplant* 2012. <https://doi.org/10.1155/2012/842141>
- Babensee JE (2008) Interaction of dendritic cells with biomaterials. *Semin Immunol* 20:101–108. <https://doi.org/10.1016/j.smim.2007.10.013>
- Bain JR, Mackinnon SE, Hudson AR et al (1988) The peripheral nerve allograft: an assessment of regeneration across nerve allografts in rats immunosuppressed with cyclosporin A. *Plast Reconstr Surg* 82:1052–1066
- Battiston B, Titolo P, Ciclamini D, Panero B (2017) Peripheral nerve defects overviews of practice in Europe. *Hand Clin* 33:545–550. <https://doi.org/10.1016/j.hcl.2017.04.005>

- Bolton EM, Bradley JA (2013) Mechanisms of immune rejection of stem cell-derived tissues: insights from organ transplantation. In: *The immunological barriers to regenerative medicine*. Springer, New York, pp 3–36
- Bradley JA, Bolton EM, Pedersen RA (2002) Stem cell medicine encounters the immune system. *Nat Rev Immunol* 2:859–871. <https://doi.org/10.1038/nri934>
- Brennan TV, Lunsford KE, Kuo PC (2010) Innate pathways of immune activation in transplantation. *J Transp Secur* 2010:1–8. <https://doi.org/10.1155/2010/826240>
- Brenner MJ, Tung TH, Jensen JN, Mackinnon SE (2002) The spectrum of complications of immunosuppression. *J Bone Jt Surgery-American* 84:1861–1870. <https://doi.org/10.2106/00004623-200210000-00020>
- Brenner MJ, Tung THH, Mackinnon SE et al (2004) Anti-CD40 ligand monoclonal antibody induces a permissive state, but not tolerance, for murine peripheral nerve allografts. *Exp Neurol* 186:59–69. <https://doi.org/10.1016/j.expneurol.2003.10.002>
- Brunelli GA, Vigasio A, Brunelli GR (1994) Invited review: different conduits in peripheral nerve surgery. *Microsurgery* 15:176–178. <https://doi.org/10.1002/micr.1920150307>
- Carvalho CR, Oliveira JM, Reis RL (2019) Modern trends for peripheral nerve repair and regeneration: beyond the hollow nerve guidance conduit. *Front Bioeng Biotechnol* 7
- Cashman CR, Hoke A (2017) Deficiency of adaptive immunity does not interfere with Wallerian degeneration. *PLoS One* 12:e0177070. <https://doi.org/10.1371/journal.pone.0177070>
- Cicciarelli JC, Lemp NA, Kasahara N (2013) Prospects for designing “universal” stem cell lines. In: *The Immunological barriers to regenerative medicine*. Springer, New York, pp 147–173
- Colvin RB, Smith RN (2005) Antibody-mediated organ-allograft rejection. *Nat Rev Immunol* 5: 807–817
- Cooper DKC, Hara H, Iwase H et al (2021) Pig kidney xenotransplantation: progress toward clinical trials. *Clin Transpl* 35:e14139. <https://doi.org/10.1111/ctr.14139>
- Davies AJ, Rinaldi S, Costigan M, Oh SB (2020) Cytotoxic immunity in peripheral nerve injury and pain. *Front Neurosci* 14:142
- Drukker M, Katz G, Urbach A et al (2002) Characterization of the expression of MHC proteins in human embryonic stem cells. *Proc Natl Acad Sci USA* 99:9864–9869. <https://doi.org/10.1073/pnas.142298299>
- Drukker M, Katchman H, Katz G et al (2006) Human embryonic stem cells and their differentiated derivatives are less susceptible to immune rejection than adult cells. *Stem Cells* 24:221–229. <https://doi.org/10.1634/stemcells.2005-0188>
- Dzhonova DV, Olariu R, Leckenby J et al (2018) Local injections of tacrolimus-loaded hydrogel reduce systemic immunosuppression-related toxicity in vascularized composite allotransplantation. *Transplantation* 1. <https://doi.org/10.1097/TP.0000000000002283>
- Evans PJ, Midha R, Mackinnon SE (1994) The peripheral nerve allograft: a comprehensive review of regeneration and neuroimmunology. *Prog Neurobiol* 43:187–233. [https://doi.org/10.1016/0301-0082\(94\)90001-9](https://doi.org/10.1016/0301-0082(94)90001-9)
- Faroni A, Mobasser SA, Kingham PJ, Reid AJ (2015) Peripheral nerve regeneration: experimental strategies and future perspectives. *Adv Drug Deliv Rev* 82–83:160–167. <https://doi.org/10.1016/j.ADDR.2014.11.010>
- Farrar CA, Kupiec-Weglinski JW, Sacks SH (2013) The innate immune system and transplantation. *Cold Spring Harb Perspect Med* 3. <https://doi.org/10.1101/cshperspect.a015479>
- Feng FY, Ogden MA, Myckatyn TM et al (2001) FK506 rescues peripheral nerve allografts in acute rejection. *J Neurotrauma* 18:217–229. <https://doi.org/10.1089/08977150150502631>
- Fisher JD, Acharya AP, Little SR (2015) Micro and nanoparticle drug delivery systems for preventing allotransplant rejection. *Clin Immunol* 160:24–35. <https://doi.org/10.1016/j.clim.2015.04.013>
- Gaudet AD, Popovich PG, Ramer MS (2011) Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. *J Neuroinflammation* 8:110
- Germain RN (1994) MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. *Cell* 76:287–299. [https://doi.org/10.1016/0092-8674\(94\)90336-0](https://doi.org/10.1016/0092-8674(94)90336-0)

- Gibson T, Medawar PB (1943) The fate of skin homografts in man. *J Anat* 77:299–310.4
- Gold BG, Storm-Dickerson T, Austin DR (1994) The immunosuppressant FK506 increases functional recovery and nerve regeneration following peripheral nerve injury. *Restor Neurol Neurosci* 6:287–296. <https://doi.org/10.3233/RNN-1994-6404>
- Gold BG, Katoh K, Storm-Dickerson T (1995) The immunosuppressant FK506 increases the rate of axonal regeneration in rat sciatic nerve. *J Neurosci* 15:7509–7516
- Gold R, Archelos JJ, Hartung H-P (2006) Mechanisms of immune regulation in the peripheral nervous system. *Brain Pathol* 9:343–360. <https://doi.org/10.1111/j.1750-3639.1999.tb00231.x>
- Gould DS, Auchincloss H (1999) Direct and indirect recognition: the role of MHC antigens in graft rejection. *Immunol Today* 20:77–82
- Gray M, Palispis W, Popovich PG et al (2007) Macrophage depletion alters the blood-nerve barrier without affecting Schwann cell function after neural injury. *J Neurosci Res* 85:766–777. <https://doi.org/10.1002/jnr.21166>
- Greenwald RJ, Freeman GJ, Sharpe AH (2005) The B7 family revisited. *Annu Rev Immunol* 23: 515–548. <https://doi.org/10.1146/annurev.immunol.23.021704.115611>
- Grinsell D, Keating CP (2014) Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. *Biomed Res Int*. <https://doi.org/10.1155/2014/698256>
- Gulati AK (1998) Immune response and neurotrophic factor interactions in peripheral nerve transplants. *Acta Haematol* 99:171–174. <https://doi.org/10.1159/000040832>
- Gulati AK, Cole GP (1994) Immunogenicity and regenerative potential of acellular nerve allografts to repair peripheral nerve in rats and rabbits. *Acta Neurochir* 126:158–164
- Hartlehnert M, Derksen A, Hagenacker T et al (2017) Schwann cells promote post-traumatic nerve inflammation and neuropathic pain through MHC class II. *Sci Rep* 7:12518. <https://doi.org/10.1038/s41598-017-12744-2>
- Hellenbrand DJ, Kaeppler KE, Ehlers ME et al (2016) Immunohistochemical assessment of rat nerve isografts and immunosuppressed allografts. *Neurol Res* 38:1094–1101. <https://doi.org/10.1080/01616412.2016.1248626>
- Hettiaratchy S, Melendy E, Randolph MA et al (2004) Tolerance to composite tissue allografts across a major histocompatibility barrier in miniature swine. *Transplantation* 77:514–521
- Ho S, Clipstone N, Timmermann L et al (1996) The mechanism of action of cyclosporin A and FK506. *Clin Immunol Immunopathol* 80:S40–S45
- Hughes R (1992) Immune responses in the peripheral nervous system. *Semin Neurosci* 4:257
- Huppa JB, Davis MM (2003) T-cell-antigen recognition and the immunological synapse. *Nat Rev Immunol* 3:973–983
- Ijkema-Paassen J, Jansen K, Gramsbergen A, Meek MF (2004) Transection of peripheral nerves, bridging strategies and effect evaluation. *Biomaterials* 25:1583–1592. [https://doi.org/10.1016/S0142-9612\(03\)00504-0](https://doi.org/10.1016/S0142-9612(03)00504-0)
- Ishida O, Ochi M, Ikuta Y, Akiyama M (1990) Peripheral nerve allograft: cellular and humoral immune responses of mice. *J Surg Res* 49:233–238. [https://doi.org/10.1016/0022-4804\(90\)90125-L](https://doi.org/10.1016/0022-4804(90)90125-L)
- Janeway CA (1989) Approaching the asymptote? Evolution and revolution in immunology. In: Cold Spring Harbor symposia on quantitative biology. Cold Spring Harbor Laboratory Press, pp 1–13
- Janeway CA (2013) Pillars article: approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb Symp Quant Biol* 1989 54:1–13. *J Immunol* 191
- Janeway CA, Bottomly K (1994) Signals and signs for lymphocyte responses. *Cell* 76:275–285
- Jensen JN, Tung THH, Mackinnon SE et al (2004) Use of anti-CD40 ligand monoclonal antibody as antirejection therapy in a murine peripheral nerve allograft model. *Microsurgery* 24:309–315. <https://doi.org/10.1002/micr.20028>
- June CH, Ledbetter JA, Linsley PS, Thompson CB (1990) Role of the CD28 receptor in T-cell activation. *Immunol Today* 11:211–216
- Kieseier BC, Hartung H-PP, Wiendl H (2006) Immune circuitry in the peripheral nervous system. *Curr Opin Neurol* 19:437–445. <https://doi.org/10.1097/01.wco.0000245365.51823.72>
- Kim BS, Yoo JJ, Atala A (2004) Peripheral nerve regeneration using acellular nerve grafts. *J Biomed Mater Res Part A* 68:201–209. <https://doi.org/10.1002/jbm.a.10045>

- Kinnear G, Jones ND, Wood KJ (2013) Costimulation blockade: current perspectives and implications for therapy. *Transplantation* 95:527–535. <https://doi.org/10.1097/TP.0b013e31826d4672>
- Klein JAN, Sato A (2000) The HLA system: first of two parts. *N Engl J Med* 343:702–709
- Kvist M, Lemplesis V, Kanje M et al (2007) Immunomodulation by costimulation blockade inhibits rejection of nerve allografts. *J Peripher Nerv Syst* 12:83–90. <https://doi.org/10.1111/j.1529-8027.2007.00126.x>
- Kvist M, Kanje M, Ekberg H et al (2008) Costimulation blockade in transplantation of nerve allografts: long-term effects. *J Peripher Nerv Syst* 13:200–207. <https://doi.org/10.1111/j.1529-8027.2008.00178.x>
- Lafferty KJ, Cunningham AJ (1975) A new analysis of allogeneic interactions. *Aust J Exp Biol Med* 53:27–42
- Längin M, Mayr T, Reichart B et al (2018) Consistent success in life-supporting porcine cardiac xenotransplantation. *Nature* 564:430–433. <https://doi.org/10.1038/s41586-018-0765-z>
- Lassner F, Schaller E, Steinhoff G et al (1989) Cellular mechanisms of rejection and regeneration in peripheral nerve allografts. *Transplantation* 48:386–392. <https://doi.org/10.1097/00007890-198909000-00006>
- Lenschow DJ, Walunas TL, Bluestone JA (1996) CD28/B7 system of costimulation. *Annu Rev Immunol* 14:233–258. <https://doi.org/10.1146/annurev.immunol.14.1.233>
- Lin MY, Manzano G, Gupta R (2013) Nerve allografts and conduits in peripheral nerve repair. *Hand Clin* 29:331–348
- Mackinnon SE, Dellon AL (1990) Clinical nerve reconstruction with a bioabsorbable polyglycolic acid tube. *Plast Reconstr Surg* 85:419–424. <https://doi.org/10.1097/00006534-199003000-00015>
- Mackinnon S, Hudson A, Falk R et al (1982) Nerve allograft response: a quantitative immunological study. *Neurosurgery* 10:61–69. <https://doi.org/10.1227/00006123-198201000-00011>
- Mackinnon SE, Midha R, Bain J et al (1992) An assessment of regeneration across peripheral nerve allografts in rats receiving short courses of cyclosporin A immunosuppression. *Neuroscience* 46:585–593
- Mackinnon SE, Doolabh VB, Novak CB, Trulock EP (2001) Clinical outcome following nerve allograft transplantation. *Plast Reconstr Surg* 107:1419–1429
- Markees TG, Phillips NE, Noelle RJ et al (1997) Prolonged survival of mouse skin allografts in recipients treated with donor Splenocytes and antibody to Cd40 Ligand1. *Transplantation* 64:329–335
- Mason D, Charlton H, Jones A et al (1986) The fate of allogeneic and xenogeneic neuronal tissue transplanted into the third ventricle of rodents. *Neuroscience* 19:685–694. [https://doi.org/10.1016/0306-4522\(86\)90292-7](https://doi.org/10.1016/0306-4522(86)90292-7)
- McLaren FH, Svendsen CN, Van der Meide P, Joly E (2001) Analysis of neural stem cells by flow cytometry: cellular differentiation modifies patterns of MHC expression. *J Neuroimmunol* 112:35–46
- Medawar PB (1944) The behaviour and fate of skin autografts and skin homografts in rabbits: a report to the war wounds Committee of the Medical Research Council. *J Anat* 78:176–199
- Midha R, Mackinnon SE, Evans PJ et al (1993) Comparison of regeneration across nerve allografts with temporary or continuous cyclosporin A immunosuppression. *J Neurosurg* 78:90–100. <https://doi.org/10.3171/jns.1993.78.1.0090>
- Midha R, Mackinnon SE, Becker LE (1994) The fate of schwann cells in peripheral nerve allografts. *J Neuropathol Exp Neurol* 53:316–322. <https://doi.org/10.1097/00005072-199405000-00013>
- Monaco S, Gehrmann J, Raivich G, Kreutzberg GW (1992) MHC-positive, ramified macrophages in the normal and injured rat peripheral nervous system. *J Neurocytol* 21:623–634. <https://doi.org/10.1007/BF01191724>
- Moore AM, Ray WZ, Chenard KE et al (2009) Nerve allotransplantation as it pertains to composite tissue transplantation. *Hand (N Y)* 4:239–244. <https://doi.org/10.1007/s11552-009-9183-x>
- Moreau A, Varey E, Anegon I, Cuturi MC (2013) Effector mechanisms of rejection. *Cold Spring Harb Perspect Med* 3

- Morris AH, Stamer DK, Kyriakides TR (2017) The host response to naturally-derived extracellular matrix biomaterials. *Semin Immunol* 29:72–91
- Mueller M, Leonhard C, Wacker K et al (2003) Macrophage response to peripheral nerve injury: the quantitative contribution of resident and hematogenous macrophages. *Lab Invest* 83:175–185. <https://doi.org/10.1097/01.LAB.0000056993.28149.BF>
- Mueller SN, Gebhardt T, Carbone FR, Heath WR (2013) Memory T cell subsets, migration patterns, and tissue residence. *Annu Rev Immunol* 31:137–161. <https://doi.org/10.1146/annurev-immunol-032712-095954>
- Norcross MA (1984) A synaptic basis for T-lymphocyte activation. *Ann l'Institut Pasteur Immunol* 135:113–134. [https://doi.org/10.1016/S0769-2625\(84\)81105-8](https://doi.org/10.1016/S0769-2625(84)81105-8)
- Oberbarnscheidt MH, Zecher D, Lakkis FG (2011) The innate immune system in transplantation. *Semin Immunol* 23:264–272
- Ochando J, Ordikhani F, Boros P, Jordan S (2019) The innate immune response to allotransplants: mechanisms and therapeutic potentials. *Cell Mol Immunol*. <https://doi.org/10.1038/s41423-019-0216-2>
- Odeberg J, Piao J-H, Samuelsson E-B et al (2005) Low immunogenicity of in vitro-expanded human neural cells despite high MHC expression. *J Neuroimmunol* 161:1–11. <https://doi.org/10.1016/j.jneuroim.2004.11.016>
- Pabari A, Lloyd-Hughes H, Seifalian AM, Mosahebi A (2014) Nerve conduits for peripheral nerve surgery. *Plast Reconstr Surg* 133:1420–1430. <https://doi.org/10.1097/PRS.0000000000000226>
- Palispis WA, Gupta R (2017) Surgical repair in humans after traumatic nerve injury provides limited functional neural regeneration in adults. *Exp Neurol* 290:106–114
- Pan D, Hunter DA, Schellhardt L et al (2019) The accumulation of T cells within acellular nerve allografts is length-dependent and critical for nerve regeneration. *Exp Neurol* 318:216–231. <https://doi.org/10.1016/j.expneurol.2019.05.009>
- Paprottka FJ, Wolf P, Harder Y et al (2013) Sensory recovery outcome after digital nerve repair in relation to different reconstructive techniques: meta-analysis and systematic review. *Plast Surg Int* 2013:704589. <https://doi.org/10.1155/2013/704589>
- Pearl JI, Lee AS, Leveson-Gower DB et al (2011) Short-term immunosuppression promotes engraftment of embryonic and induced pluripotent stem cells. *Cell Stem Cell* 8:309–317. <https://doi.org/10.1016/j.stem.2011.01.012>
- Pearson TC, Alexander DZ, Winn KJ et al (1994) Transplantation tolerance induced by CTLA4-Ig1. *Transplantation* 57:1701–1705
- Peltonen S, Alanne M, Peltonen J (2013) Barriers of the peripheral nerve. *Tissue Barriers* 1:e24956. <https://doi.org/10.4161/tisb.24956>
- Peugh WN, Superina RA, Wood KJ, Morris PJ (1986) The role of H-2 and non-H-2 antigens and genes in the rejection of murine cardiac allografts. *Immunogenetics* 23:30–37
- Phillips LK, Gould EA, Babu H et al (2013) Natural killer cell-activating receptor NKG2D mediates innate immune targeting of allogeneic neural progenitor cell grafts. *Stem Cells*. <https://doi.org/10.1002/stem.1422>
- Pollard JD, Westland KW, Harvey GK et al (1995) Activated T cells of nonneural specificity open the blood-nerve barrier to circulating antibody. *Ann Neurol* 37:467–475. <https://doi.org/10.1002/ana.410370409>
- Powell HC, Olee T, Brostoff SW, Mizisin AP (1991) Comparative histology of experimental allergic neuritis induced with minimum length neuritogenic peptides by adoptive transfer with sensitized cells or direct sensitization. *J Neuropathol Exp Neurol* 50:658–674. <https://doi.org/10.1097/00005072-199109000-00010>
- Ray WZ, Kale SS, Kasukurthi R et al (2011) Effect of cold nerve allograft preservation on antigen presentation and rejection. *J Neurosurg* 114:256–262. <https://doi.org/10.3171/2010.5.JNS10111>
- Raza C, Riaz HA, Anjum R, Shakeel N ul A (2020) Repair strategies for injured peripheral nerve: review. *Life Sci* 243:117308
- Rezzani R (2006) Exploring cyclosporine A-side effects and the protective role-played by antioxidants: the morphological and immunohistochemical studies. *Histol Histopathol* 21:301–316

- Richner M, Ferreira N, Dudele A et al (2019) Functional and structural changes of the blood-nerve barrier in diabetic neuropathy. *Front Neurosci* 12:1038. <https://doi.org/10.3389/fnins.2018.01038>
- Roballo KCS, Bushman J (2019) Evaluation of the host immune response and functional recovery in peripheral nerve autografts and allografts. *Transpl Immunol* 53:61–71. <https://doi.org/10.1016/j.TRIM.2019.01.003>
- Rock KL, Kono H (2007) The inflammatory response to cell death. <https://doi.org/10.1146/annurev.pathmechdis.3.121806.151456>
- Roopenian D, Young Choi E, Brown A (2002) The immunogenomics of minor histocompatibility antigens. *Immunol Rev* 190:86–94
- Rotshenker S (2011) Wallerian degeneration: the innate-immune response to traumatic nerve injury. *J Neuroinflammation* 8:109109
- Rustemeyer J, van de Wal R, Keipert C, Dicke U (2010) Administration of low-dose FK 506 accelerates histomorphometric regeneration and functional outcomes after allograft nerve repair in a rat model. *J Cranio-Maxillofacial Surg* 38:134–140. <https://doi.org/10.1016/j.JCMS.2009.03.008>
- Safinia N, Scotta C, Vaikunthanathan T et al (2015) Regulatory T cells: serious contenders in the promise for immunological tolerance in transplantation. *Front Immunol* 6:438
- Sakaguchi S (2000) Regulatory T cells: key controllers of immunologic self-tolerance. *Cell* 101:455–458
- Sandrin MS, Vaughan HA, Dabkowski PL, McKenzie IFC (1993) Anti-pig IgM antibodies in human serum react predominantly with Gal(α 1-3)Gal epitopes. *Proc Natl Acad Sci USA* 90:11391–11395. <https://doi.org/10.1073/pnas.90.23.11391>
- Santos Roballo KC, Dhungana S, Jiang Z et al (2019) Localized delivery of immunosuppressive regulatory T cells to peripheral nerve allografts promotes regeneration of branched segmental defects. *Biomaterials* 209:1–9. <https://doi.org/10.1016/j.biomaterials.2019.04.015>
- Sayegh MH, Turka LA (1998) The role of T-cell costimulatory activation pathways in transplant rejection. *N Engl J Med* 338:1813–1821
- Schneider SC, Sercarz EE (1997) Antigen processing differences among APC. *Hum Immunol* 54:148–158
- Schwartz RH (1990) A cell culture model for T lymphocyte clonal anergy. *Science* (80–) 248:1349–1356
- Sefton MV, Babensee JE, Woodhouse KA (2008) Innate and adaptive immune responses in tissue engineering. *Semin Immunol* 20:83–85. <https://doi.org/10.1016/j.smim.2007.12.008>
- Shamash S, Reichert F, Rotshenker S (2002) The cytokine network of wallerian degeneration: tumor necrosis factor- α , interleukin-1 α , and interleukin-1 β . *J Neurosci* 22:3052–3060. <https://doi.org/10.1523/jneurosci.22-08-03052.2002>
- Siemionow M, Sonmez E (2007) Nerve allograft transplantation: A review. *J Reconstr Microsurg* 23:511–520. <https://doi.org/10.1055/s-2007-1022694>
- Singer B, King L, D'Alessio F (2014) Regulatory T Cells as immunotherapy. *Front Immunol* 5:46
- Siu JHY, Surendrakumar V, Richards JA, Pettigrew GJ (2018) T cell allorecognition pathways in solid organ transplantation. *Front Immunol* 9:2548
- Spies JM, Westland KW, Bonner JG, Pollard JD (1995) Intraneural activated t cells cause focal breakdown of the blood-nerve barrier. *Brain* 118:857–868. <https://doi.org/10.1093/brain/118.4.857>
- Stoll G, Griffin JW, Li CY, Trapp BD (1989) Wallerian degeneration in the peripheral nervous system: participation of both Schwann cells and macrophages in myelin degradation. *J Neurocytol* 18:671–683. <https://doi.org/10.1007/BF01187086>
- Tai CY, Weber RV, Mackinnon SE, Tung TH (2010) Multiple costimulatory blockade in the peripheral nerve allograft. *Neurol Res* 32:332–336. <https://doi.org/10.1179/174313209X385635>
- Taylor PA, Noelle RJ, Blazar BR (2001) Cd4+Cd25+ immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade. *J Exp Med* 193:1311–1318. <https://doi.org/10.1084/jem.193.11.1311>

- Taylor CJ, Peacock S, Chaudhry AN et al (2012) Generating an iPSC bank for HLA-matched tissue transplantation based on known donor and recipient hla types. *Cell Stem Cell* 11:147–152. <https://doi.org/10.1016/j.stem.2012.07.014>
- Teh LK, Dom SHM, Zakaria ZA, Salleh MZ (2011) A systematic review of the adverse effects of tacrolimus in organ transplant patients. *African J. Pharm. Pharmacol.* 5:764–771
- Trombetta ES, Mellman I (2005) Cell biology of antigen processing in vitro and in vivo. *Annu Rev Immunol* 23:975–1028. <https://doi.org/10.1146/annurev.immunol.22.012703.104538>
- Udina E, Voda J, Gold BG, Navarro X (2003) Comparative dose-dependence study of FK506 on transected mouse sciatic nerve repaired by allograft or xenograft. *J Peripher Nerv Syst* 8:145–154. <https://doi.org/10.1046/j.1529-8027.2003.03020.x>
- Waller A (1850) XX. Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibres. *Philos Trans R Soc London* 140:423–429. <https://doi.org/10.1098/rstl.1850.0021>
- Walunas TL, Lenschow DJ, Bakker CY et al (1994) CTLA-4 can function as a negative regulator of T cell activation. *Immunity* 1:405–413. [https://doi.org/10.1016/1074-7613\(94\)90071-X](https://doi.org/10.1016/1074-7613(94)90071-X)
- Weerasuriya A, Hockman CH (1992) Perineurial permeability to sodium during Wallerian degeneration in rat sciatic nerve. *Brain Res* 581:327–333. [https://doi.org/10.1016/0006-8993\(92\)90727-Q](https://doi.org/10.1016/0006-8993(92)90727-Q)
- Wekerle H, Schwab M, Linington C, Meyermann R (1986) Antigen presentation in the peripheral nervous system: schwann cells present endogenous myelin autoantigens to lymphocytes. *Eur J Immunol* 16:1551–1557. <https://doi.org/10.1002/eji.1830161214>
- Wekerle H, Sun D, Oropeza-Wekerle RL, Meyermann R (1987) Immune reactivity in the nervous system: modulation of T-lymphocyte activation by glial cells. *J Exp Biol* 132:43–57
- Whitlock EL, Tuffaha SH, Luciano JP et al (2009) Processed allografts and type I collagen conduits for repair of peripheral nerve gaps. *Muscle Nerve* 39:787–799. <https://doi.org/10.1002/mus.21220>
- Wood KJKJJ, Goto R (2012) Mechanisms of rejection: current perspectives. *Transplantation* 93:1. <https://doi.org/10.1097/TP.0b013e31823cab44>
- Yan Y, Sun HH, Hunter DA et al (2012) Efficacy of short-term FK506 administration on accelerating nerve regeneration. *Neurorehabil Neural Repair* 26:570–580. <https://doi.org/10.1177/1545968311431965>
- Yatim KM, Lakkis FG (2015) A brief journey through the immune system. *Clin J Am Soc Nephrol* 10:1274 LP–1281. <https://doi.org/10.2215/CJN.10031014>
- Yoshida M, Babensee JE (2004) Poly(lactic-co-glycolic acid) enhances maturation of human monocyte-derived dendritic cells. *J Biomed Mater Res Part A* 71:45–54. <https://doi.org/10.1002/jbm.a.30131>
- Zalewski AA, Silvers WK (1980) An evaluation of nerve repair with nerve allografts in normal and immunologically tolerant rats. *J Neurosurg* 52:557–563. <https://doi.org/10.3171/jns.1980.52.4.0557>
- Zalewski AA, Kadota Y, Azzam NA, Azzam RN (1993) Observations on the blood and perineurial permeability barriers of surviving nerve allografts in immunodeficient and immunosuppressed rats. *J Neurosurg* 78:794–806. <https://doi.org/10.3171/jns.1993.78.5.0794>
- Zhao T, Zhang ZN, Rong Z, Xu Y (2011) Immunogenicity of induced pluripotent stem cells. *Nature* 474:212–215. <https://doi.org/10.1038/nature10135>
- Zheng D, Wang X, Xu R-H (2016) Concise review: one stone for multiple birds: generating universally compatible human embryonic stem cells. *Stem Cells* 34:2269–2275. <https://doi.org/10.1002/stem.2407>
- Zhong R (2007) Gal knockout and beyond. *Am J Transplant* 7:5–11. <https://doi.org/10.1111/j.1600-6143.2006.01615.x>
- Zhou W, Medof ME, Heeger PS, Sacks S (2007) Graft-derived complement as a mediator of transplant injury. *Curr Opin Immunol* 19:569–576
- Zuo KJ, Shafa G, Chan K et al (2021) Local FK506 drug delivery enhances nerve regeneration through fresh, unprocessed peripheral nerve allografts. *Exp Neurol* 113680. <https://doi.org/10.1016/j.expneurol.2021.113680>



Autonomic Nervous System Repair and Regeneration

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Abstract

The autonomic nervous system is a component of the peripheral nervous system involved in visceral functions and divided into three distinct components: sympathetic, parasympathetic, and enteric nervous system. All these divisions are made up of afferent and efferent fibers providing sensory input to the central nervous system and motor output through motor visceral nerves.

Similarly to the somatic nervous system, lesions affecting the autonomic nervous system are very frequent and, depending on the degree of severity, result in a complete or partial loss of functionality of the visceral organ involved. Given the anatomical location, most autonomic nerve lesions are classified as iatrogenic injuries caused by possible complications in various medical treatments, drug administration, injection, radiation, traction during preoperative patient positioning, and surgical procedures. This review provides an overview of the most frequent iatrogenic autonomic nerve lesions occurring during substantial surgical procedures, prostatectomy, hysterectomy, and heart transplantation. In addition, the main factors that influence and characterize nerve regeneration in the autonomic nervous system will be discussed and a particular focus on the importance of in vitro and ex vivo models, which allow extrapolation of important data in the study of such regeneration, will be provided. Finally, an overview of the main scientific evidence that testifies to neurogenesis in the enteric nervous system will be provided, where some changes, following several types of nerve damage and injuries, in cell signaling molecules are thought to have neurogenic properties in the gut.

1 Introduction

The autonomic nervous system (ANS) is a component of the peripheral nervous system involved in visceral functions controlling organs of the thoracic, abdominal, and pelvic cavities. The ANS affects heart rate, digestion, respiratory rate, salivation, pupillary dilation, as well as urinary and sexual functions. It comprises three distinct divisions: sympathetic (SNS), parasympathetic (PSNS), and enteric (ENS) (Furness 2006).

The SNS and PNS are both made up of afferent and efferent fibers that provide sensory input and motor output, respectively, to the central nervous system (CNS) (Waxenbaum and Varacallo 2019). Basically the motor pathways of SNS and PSNS consist of two-neuron series: a preganglionic neuron with a cell body in the CNS and a postganglionic neuron with a cell body in the periphery that supplies innervation to target organs (Waxenbaum and Varacallo 2019). The ENS innervates the gastrointestinal tract containing over 100 million neurons of over 15 morphologies, is

responsible for the regulation of digestive processes, and is connected through the vagus nerve to the central nervous system (CNS) (Belkind-Gerson et al. 2006).

2 Sympathetic Nervous System

The sympathetic nervous system (SNS) affects the peripheral transmission of the visceral organs through nerves and ganglia. Preganglionic neurons are located in the thoracolumbar region of the spinal cord at T1 to L2–L3 level. Preganglionic nerve fibers reach the paravertebral ganglia and synapse with postganglionic neurons. From there, the long postganglionic neuron axons traverse most of the body reaching different target organs. Basically one preganglionic neuron may synapse with 15–20 postganglionic neurons, allowing the wide diffusion of many autonomic effects (Fig. 1). Preganglionic axons are myelinated with an organization that resembles the somatic nerves, while postganglionic axons are unmyelinated and organized in small-diameter bundles surrounded by a single Schwann cell (Geuna et al. 2018). The SNS is involved in responses that would be associated with fighting or fleeing, they reach the viscera causing vasoconstriction, bronchial and bronchiolar dilatation,

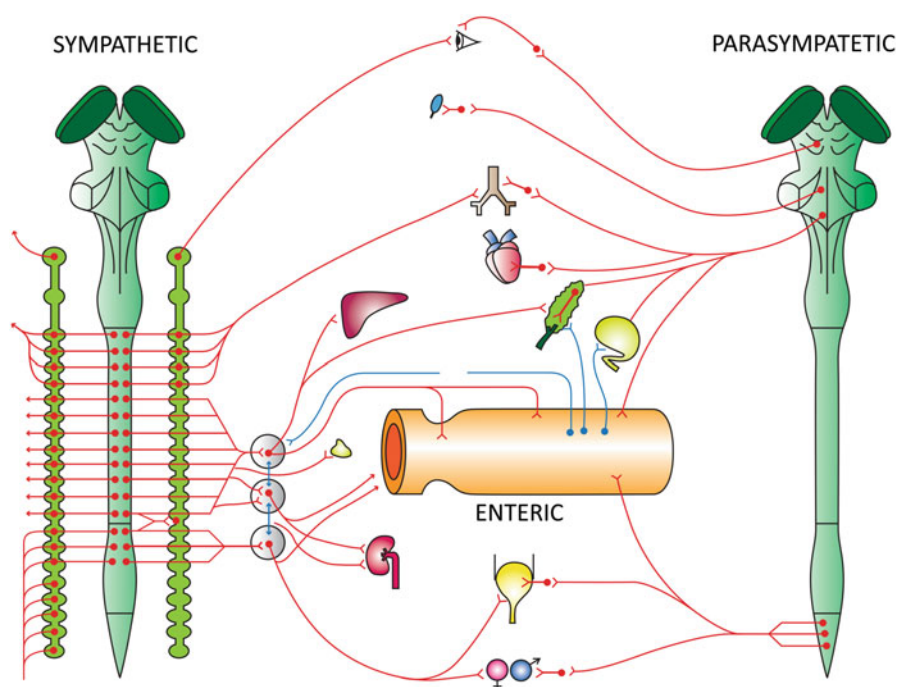


Fig. 1 Representation of autonomic nervous system anatomy: sympathetic, parasympathetic, and enteric division

papillary dilatation, inhibition of gastrointestinal muscle contraction, etc. (Furness 2006; Saper 2002; Swenson 2006).

3 Parasympathetic Nervous System

Preganglionic parasympathetic neurons are located in cranial nerve nuclei of the brainstem and in the grey matter of the second to fourth sacral segments of the spinal cord. Postganglionic parasympathetic neurons are located distant from the CNS, in ganglia near the innervated structure; in the wall of viscera, their fibers are usually unmyelinated and shorter than the sympathetic fibers (Geuna et al. 2018).

Parasympathetic preganglionic neurons are located in brainstem nuclei arising with cranial nerves III, VII, IX, and X, termed the “craniosacral outflow” and with spinal nerve in sacrum, termed “the sacral segments” (S2–S4). The vagus nerve supplies innervations to thoracic and abdominal organs and synapse with ganglia very close to (or within) the organ walls (Fig. 1). The pelvic parasympathetic segments activate bladder contraction and also supply lower abdominal and pelvic organ innervation (Furness 2006).

4 Enteric Nervous System

The enteric nervous system (ENS) is the most extensive branch of the peripheral nervous system that provides innervation of the gut, consisting of two layers of ganglia and fibers encircling the gastrointestinal tract (Lake and Heuckeroth 2013). The ENS regulates the proper functioning of the gastrointestinal tract controlling smooth muscles activities and fluid secretion; these processes allow the disruption of macroscopic food particles, the extraction of nutrients, and the maintenance of a healthy luminal microbiome. The ENS comprises a network of neurons and glial cells within the wall of the bowel that contains more than 15 functional classes of neurons, approximately the same number located in the adult spinal cord, with a wide range of neurotransmitters, projection patterns, and electrical properties (Belkind-Gerson et al. 2006; Furness 2006; Walsh and Zemper 2019). Furthermore, many thousands of ganglia compose the ENS, mainly located in two plexuses, the myenteric and submucosal; within both plexuses, neuronal cell bodies reside in ganglia surrounded by glia.

The myenteric plexus is anatomically located between the longitudinal and circular muscle layers and coordinates motility of the gut including neurons of the parasympathetic and sympathetic branches of the peripheral nervous system. Nerve fibers and ganglia form a continuous network extending from the upper esophagus to the internal anal sphincter.

The submucosal plexus is located above the circular muscle layer close to the mucosa, and regulates secretory activity of the gut: Submucosal ganglia and connecting nerve fibers are mainly in the small and large intestines, while extremely

rare in the stomach and esophagus; small ganglia are located also in the mucosa layer, close to the muscularis mucosae (Hu and Spencer 2018).

Neuronal projections supply innervations of individual ganglia to each other and to the enteric epithelium. Particularly, neurons located outside the intestine account for extrinsic innervations and comprise nerves from parasympathetic and sympathetic branches, most of them enter the intestine through the vagus nerve. Intrinsic innervation arises from neurons with cell bodies within the intestine (Belkind-Gerson et al. 2006; Walsh and Zemper 2019).

5 Peripheral Nerve Injury Classification

Autonomic nerve injury may occur as a consequence of several clinical conditions associated to partial or complete loss of motor or sensory function, leading to long-term disability (Grinsell and Keating 2014). In 1942, Sir Herbert Seddon established a classification with three types of nerve injury: neurapraxia, axonotmesis, and neurotmesis according to lesion severity (Geuna et al. 2009; Seddon 1942).

Neurapraxia is the milder injury in which axons are undamaged but myelin disruption occurs; functional recovery is basically faster taking place within hours up to a few months.

Axonal damage with integrity of connective tissue identifies the second type of Seddon's injury, the "axonotmesis"; in this case nerve fiber regeneration and functional recovery are influenced by several factors such as the distance from the target organ.

The most severe nerve injury according to Seddon's classification is the "neurotmesis" characterized by complete nerve fiber disruption. This type of lesion requires a surgical procedure to promote nerve repair.

In 1951, Sunderland split Seddon's classification in further degrees based on the connective tissue involvement (Sunderland 1951). According to Sunderland's classification, five types of lesion have been proposed in which Type 1 and Type 2 correspond to Seddon's neurapraxia and axontomesis, respectively; Sunderland Type 3 is characterized by injured endoneurium with intact perineurium and epineurium; in Sunderland Type 4 nerve fascicle transection occurs with intact epineurium. Lastly, in Type 5 injury (Seddon's neurotmesis) complete nerve disruption takes place and spontaneous recovery is negligible.

Since nerve may undergo a combination of different degrees of injury, Sunderland grading is more often used by surgeons in order to decide the type of surgical procedure for nerve repair. In 1988, Susan E. Mackinnon and A. Lee Dellon describe a further degree of nerve injury based on a mixed type of nerve injury (Mackinnon 1989).

6 Axon Regrowth in the Autonomic Nervous System

After injury to autonomic nerves, several mechanisms occur at the injury site almost immediately including molecular and morphological changes that occur both proximally and distally to the lesion site (Geuna et al. 2018).

During the initial post-injury phase, axons distal to the lesion site are disconnected from the neuronal body and neurons react to axonal damage switching from a transmitter to a regenerative state: several genes are upregulated as well as the synthesis of molecules normally not expressed in adult neurons. These changes activate and regulate neuronal pathways responsible for survival and axonal regeneration (Kreutzberg 2009; Navarro et al. 2007) (Fig. 2).

While neuronal soma and proximal axons are preparing for growth, axons distal to the lesion site undergo “Wallerian degeneration,” considered the key step for the regenerative process (Gaudet et al. 2011). During this crucial step, Schwann cells change their normal phenotype responsible for myelination in the case of preganglionic axons, or glial winding in postganglionic unmyelinated axons, and start proliferating and transdifferentiating.

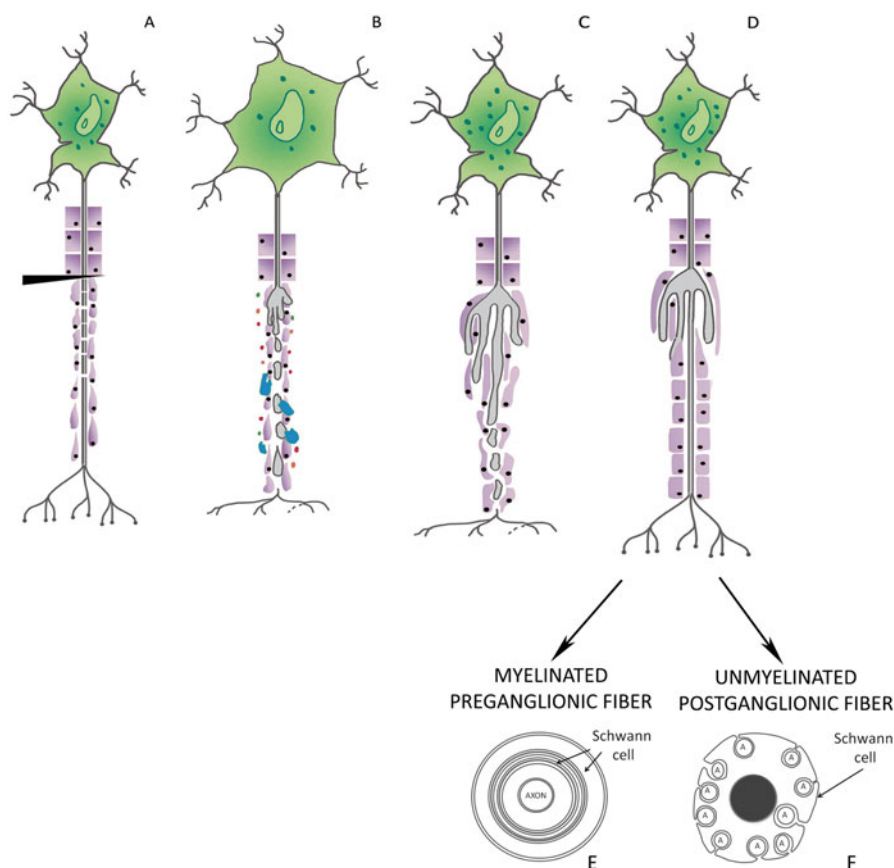


Fig. 2 Nerve fibers regeneration pathway: (a) injured nerve fiber; (b) Wallerian degeneration; (c) bands of Büngner formation and axonal elongation; (d) reinnervation of target organ; (e) myelination in the case of preganglionic nerve fibers; and (f) glial winding in postganglionic axons

Indeed, growth-promoting genes encoding cell adhesion molecules and growth factors are upregulated. Proliferating Schwann cells arrange themselves in columns known as “bands of Büngner” that guide the regenerating axons (Kim et al. 2013). The functional importance of the regeneration process in ANS is to reach the target visceral organ in order to restore normal organ functions.

7 Clinical Iatrogenic Damage Affecting Autonomic Nerves

Iatrogenic nerve injuries are considered as a potential complication in various medical treatments including drug administration, anesthetic injection, radiation, antitubercular drugs, traction during preoperative patient positioning, and surgical procedures (Antoniadis et al. 2014; Moore et al. 2012). The most common accidental nerve damage occurs during surgical intervention in which nerves can be cut, stretched, and ligated leading to morbidity and impairment in the patient's quality of life. It has been reported that up to 17.4% of all nerve injuries occur as a consequence of iatrogenic lesions (Carter et al. 2020). In the following sections, the main iatrogenic autonomic nerve damage affecting visceral organs will be described.

8 Heart

The intact heart is innervated by fibers of the ANS, parasympathetic and sympathetic (Grupper et al. 2018). The medulla is the control station for the regulation of autonomic information addressed to the heart and blood vessels. It in turn is regulated by the hypothalamus and the effect is visible at the level of cardiovascular responses to stress and emotions. The vagus nerve, with its three nuclei in the central nervous system (CNS), 1) the dorsal motor nucleus, 2) the ambiguus nucleus, and 3) the solitary nucleus, controls the cardiovascular system.

Heart transplantation (HTx) provokes an interruption of the parasympathetic vagal neurons and of the intrinsic postganglionic sympathetic nerve fibers that come from the stellate ganglia to reach the myocardium, causing axonal Wallerian degeneration and cardiac denervation (Grupper et al. 2018; Norvell and Lower 1973). Interruption of nerve signalling provokes the disappearance of input to the sinoatrial node and the loss of efferent and afferent nerve signaling toward and out of the cardiac organ (Braith et al. 1992; Hayman et al. 2010). Axonal degeneration completes within a few days of transplantation and leads to a total lack of cardiac reception of noradrenaline, so to a complete altered control of the cardiovascular system (Awad et al. 2016). Thus cardiovascular homeostasis is completely altered, with impairment of the vasoregulatory response to changes in cardiac filling pressure.

Exercise capacity in heart transplant recipients is less efficient due to the fact that hearts that have undergone denervation rely only on the circulation for the release of catecholamines (Bengel et al. 2001).

In fact cardiac transplantation, resulting in total denervation of the donor heart, also includes the sinus node, that is the control unit of the heart rate (Cooper et al. 1962; Stinson et al. 1972). Plasma catecholamine concentrations are responsible for any further increases in heart rate during exercise and, after cessation, the persistence of this increase is due to a delayed humoral catecholamine release. Wilson and colleagues demonstrated that sympathetic reinnervation after human cardiac transplantation restores the levels of left ventricular norepinephrine (Wilson et al. 2000).

A large literature reports the late onset of cardiac reinnervation in a percentage of patients ranging from 40–70% after HTx (Buendia-Fuentes et al. 2011; Lovric et al. 2004; Parry et al. 1997). The use of microscopy made it possible to monitor the regrowth of the sympathetic innervation that occurs along the coronary blood vessels and the anastomoses between the donor heart and the recipient portions (Wilson et al. 1991). A vast literature reports the reinnervation modality after HTx, which is heterogeneous, i.e., it appears only partially and regionally, and a transmural heterogeneity of the nervous distribution is reported (Doering et al. 1996; Kaye et al. 1993; Überfuhr et al. 2000a, b).

9 Prostate

Prostate cancer (PCa) is the most common cancer among the male population (Boyle and Ferlay 2005; Jemal et al. 2008a; Ahmedin Jemal et al. 2008b). Currently the gold standard treatment for prostate cancer is the radical prostatectomy that involves the removal of all gland. Despite many advancements in the urological surgical field that have been established, such as the 3D high-definition vision system and the use of a robotic system to perform minimal invasive surgery (Porpiglia et al. 2018), many patients are affected by erectile dysfunction after radical prostatectomy due to iatrogenic damage to the autonomic peri-prostatic nerve bundles (Geuna et al. 2013).

In order to improve the regeneration of the periprostatic nerve and the functional recovery, different strategies have been proposed such as the application of different biomaterials as devices to enhance axonal regeneration. Particularly, the application of two different membranes is proved to be useful to protect and enhance the regeneration process of the periprostatic nerve inside the neurovascular bundle surrounding the prostatic gland (Geuna et al. 2013). Indeed, several studies reported the clinical use of dehydrated human amnion/chorion membranes (dHacM) on the neurovascular bundles after nerve sparing radical prostatectomy as a neuroprotective, pro-regenerative, and anti-inflammatory device (Patel et al. 2015; Quinlan et al. 1989).

Interestingly, membranes made of a biomaterial of natural origin such as “chitosan” have been reported to be safe and effective in the urological clinical field (Geuna et al. 2013). The regenerative potential of a chitosan membrane was tested for the first time in ex vivo organotypic autonomic explant ganglia cultures showing significant neurite elongation on chitosan substrate (Muratori et al. 2019). Furthermore, clinical results obtained by Porpiglia et al. (2018) reported a clinical trial in which the chitosan membrane is able to promote nerve regeneration as a

protective device for neurovascular bundles following bilateral nerve sparing radical prostatectomy (Geuna et al. 2013; Porpiglia et al. 2018).

10 Uterus

Cervical cancer ranks second among the most common cancers in women with 500,000 new cases and 274,000 deaths per year. In the case of early-stage cervical cancer, the gold standard treatment is radical hysterectomy (RH) with complete bilateral pelvic lymphadenectomy (Verleye et al. 2009). RH consists of explantation of the uterus, cervix, and the upper part of the vagina (Querleu and Paul Morrow 2008). Surgical practice is the cause of iatrogenic nerve damages that come to life following events such as coagulation, stretching, resection, or intraoperative organ displacement. The disfunctions can be urinary (hypocontractile or a contractile bladder) (Sekiyama et al. 2020), on the digestive and sexual system (Fujii 2008).

Briefly, the innervation of the pelvic district is represented by the superior hypogastric plexus, the hypogastric nerves, and the sacral splanchnic nerves, all characterized by the sympathetic connotation and the parasympathetic pelvic splanchnic nerves. The union of these nerves leads to the formation of the lower hypogastric plexus which with its efferences reaches the uterus, vagina, urinary bladder, and rectum (Fujii 2008; Magrina et al. 2021).

A great effort has been made to ensure a correct classification of the different procedures included in the definition of radical hysterectomy despite having different degrees of radicality (Querleu et al. 2017; Querleu and Paul Morrow 2008). There are four levels, they also maintain several sublevels and are the following:

- Type A – limited radical hysterectomy. The objective of the intervention is to provide the complete removal of the cervix below the vaginal fornix, with a paracervical margin.
- Type B – resection of the paracervix at the ureter. The ureter is mobilized laterally, to excise the paracervix at the level of the ureteral tunnel.
- Type C – transection of the paracervix at the level of the internal iliac vascular system with/without preservation of autonomic nerves. This is the classical radical hysterectomy.
- Type D – laterally extended resection. These are ultraradical procedures, in which all the structures laterally located with respect to the paracervix are resected.

Several experimental works performed on preclinical models had the intent to assess the extent of nerve damage that follows an RH and the functional deficit that provokes. To simulate RH-induced neurapraxia Hannan and colleagues have developed a particular model of crush, a nerve crush technique aimed at nerve supplying the vagina and bladder in female rats (Hannan et al. 2017). The bilateral nerve injury, the lesion model representing RH, caused a dramatic bladder size augmentation with

a decreased smooth muscle and autonomic input, but importantly a return to sham condition was observed after 30 days. In another experimental study, in which characterization of the model of nerve injury was performed to test the functionality of the destrusor muscle, with a bilateral pelvic nerve crush injury (BPNI), a bladder decompensation with incontinence and a consequent partial functional recovery at 3 and 9 weeks were observed (Dewulf et al. 2020).

11 Factors Influencing Regeneration of the Autonomic Nervous System

The ANS innervates all tissues different from skeletal muscles, which are innervated by somatic nerves, and it controls, mainly through reflexes, the vital body functions (Jiman et al. 2020). Even if the autonomic system is composed of neurons with many shared features with somatic neurons there are differences between those two categories, such as molecules and neuropeptides produced during synaptic communication, like norepinephrine (Donadio et al. 2019; McCorry 2007). Even if neurons of ANS show remarkable differences compared with somatic neurons and neuronal populations, the nerve structure is very similar as shown by Yan and coworkers that used a vagus nerve graft after a musculocutaneous nerve injury with effective results comparable to the gold standard intervention in nerve injury repair, the end-to-end repair technique (Yan et al. 2017).

ANS recovery is influenced by different factors, the site of injury, the integrity of the connective tissue surrounding the injured axons, and the formation of the intraneural scar (Wang et al. 2019). ANS is composed of C and A δ fibers, in particular, the preganglionic axons are lightly myelinated, postganglionic axons are unmyelinated, and they differ from somatic nerves, which are composed of large-diameter myelinated and small unmyelinated fibers. For this reason regeneration is faster in autonomic than somatic nerve; autonomic nerve regeneration rate is approximately 3 mm/day (Archer and McLean 1988; McLean 1985). Also, the ANS regeneration more often leads to complete functional recovery, a condition that is very rare after somatic nerve injuries (Fu and Gordon 1995; Sulaiman and Gordon 2013). Similar to peripheral somatic nerve injuries, recovery time is longer in the case of more proximal injury from the neuronal cell body (Menorca et al. 2013; Navarro 2016).

Studies on vagal nerve regeneration after partial or complete damage have been reported since 1771 in animal models (Cameron 1933). It has also been established by McRae and colleagues that the ciliary neurotrophic factor (CNTF) has the greatest positive effect on autonomic motor neuron outgrowth (McRae et al. 2012). Instead, the enteric autonomic nervous system regeneration processes are strongly promoted by the glial cell line-derived neurotrophic factor (GDNF) due to the activation of c-Jun N-terminal kinase and Src kinase (Blennerhassett and Lourenssen 2021). The drugs tested in the last two decades like midodrine (Lin et al. 2003; Parsaik et al. 2013; Schrage et al. 2004) seem to have small to null beneficial effects in promoting ANS regeneration.

12 Experimental Models to Study Autonomic Nervous System Regeneration

Since the ANS plays a key role in maintaining vital functions, several experimental strategies are currently used to study the autonomic regeneration processes comprising *in vitro* and *ex vivo* models (Krenz and Weaver 1998; Wu and Zeltner 2020).

In particular, *in vitro* models are suitable to study selective cellular pathways and the PC-12 cell line is a validated model to assess the autonomic neuron responses to several enteric dysfunctions, systemic autonomic genetic, and acquired diseases (Van Bergeijk et al. 2006; Namkoong et al. 2017; Sharifi et al. 2007; Verma et al. 2019). The PC-12 cell line derives from a pheochromocytoma of the rat adrenal medulla and had an embryonic origin from the neural crest. This cell line is a validated autonomic neuronal model due to its selective ability to release epinephrine, a neurotransmitter characterizing neurons of the ANS (Greene et al. 1976).

Several studies have addressed the need to find an *in vitro* model of autonomic neurons through the differentiation of pluripotent stem cells to study autonomic diseases like familial dysautonomia, pure autonomic failure, and post-injury ANS regeneration, but these studies are still mostly preliminary and ongoing (Donadio et al. 2019; Krenz and Weaver 1998).

Interestingly, other suitable models to study the regeneration of ANS are *ex vivo* explants from vagus, cervical and prostatic ganglia harvested from adult male rats (Muratori et al. 2019; Odom et al. 2018; Randolph et al. 2020).

The advantage of adopting the explant ganglia model is the possibility to assess the autonomic regeneration processes at the cellular level, reducing the number of sacrificed animals with more translatability to clinical settings (Flecknell 2002). Indeed, autonomic ganglia explants keep the cytoarchitecture of the organ they are derived from and the communications between neurons, glial, and connective tissue cells are preserved (Fregnan et al. 2016; Muratori et al. 2019). In addition, they are models suitable to study acute autonomic nerve injury and early stages of nerve regeneration, since they can be cultured for at most 1 week. Furthermore, prostatic explant ganglia are suitable to study the toxic effects of radiation therapy on autonomic neurons responsible for erectile dysfunction. In particular, Randolph and colleagues have discovered that the whole ganglia explants have a protective effect in avoiding cell apoptosis and promoting nerve regeneration compared to prostatic dissociated ganglia (Randolph et al. 2020). For those reasons, autonomic explant ganglia are *ex vivo* models able to bridge the gap between cell lines and *in vivo* experiments.

13 Postnatal Neurogenesis in the Autonomic Nervous System

In the enteric nervous system (ENS) postnatal neurogenesis may occur as a consequence of certain types of injury; several studies suggest that neurogenesis occurs perpetually throughout life (Belkind-Gerson et al. 2015; Laranjeira et al. 2011). The human gut contains approximately 500 million neurons that form a complex

innervation considered to be largely independent of central nervous system (CNS) input (Gabella 1987). The ENS is involved in gut motility, regulation of blood flow, nutrient absorption, and immune function of the gut (Furness 2008; Genton and Kudsk 2003; Laranjeira et al. 2011). In case of inflammation, injury, and other types of injury-related damages, ENS functions can be impaired (Jonscher and Belkind-Gerson 2019). These conditions are related to changes in molecules responsible of cell signaling such as GDNF, serotonin (5-hydroxytryptamine [HT]), endocannabinoids, and bacterial lipopolysaccharide (LPS) that are thought to have neurogenic properties in the gut (Von Boyen et al. 2011; Schuster et al. 2014; Storr et al. 2008). It has been demonstrated that these events lead to neuronal death, but upon restoration of physiologic function, it has been shown a partial recover in the neuronal number, index of compensatory neurogenesis (Belkind-Gerson et al. 2015; Hanani et al. 2003; Laranjeira et al. 2011).

The possible occurrence of postnatal neurogenesis in the ENS system has been postulated since the beginning of the twentieth century. Already in 1913, the postnatal histogenesis of myenteric neurons was reported by Miura in the rat small intestine in pathological conditions (Miura 1913). Later on, Ambrosi showed the occurrence of an increase of the number of rat myenteric neurons after a short-term intestinal occlusion as a consequence of differentiation of resident neuronal precursors (Ambrosi 1942). In 1951, Bennighoff investigated the rat myenteric ganglia in the loops upstream from an intestinal stenosis and reported an increase in the size and number of neurons of the Auerbach's and Meissner's plexuses (Benninghoff 1951). A few years later Benninghoff's experiment was repeated by Filogamo and Vigliani (1953, 1954) in dogs, showing that both neuronal hypertrophy and hyperplasia occurred in the gut tract upstream from a partial stenosis. These authors postulated that, due to the absence of mitotic neural cells, the increase in the number of neurons should be assigned to poorly differentiated or undifferentiated cells persisting in adult myenteric plexus and remaining capable to turn into neurons under exceptional stimuli. Neurogenesis leads to an increase of four- to fivefold in the number of mature neurons giving rise to new small enteric ganglia.

These authors also demonstrated that neuronal hypertrophy, but not hyperplasia, is a reversible process showing that intestinal hypotrophy in the loops upstream from the stenosis by means of the Thiry–Vella procedure (the surgical exclusion of an intestinal loop from the transit by connection to the abdominal wall) induced a marked decrease only in neural cell size but not in neuronal number (Filogamo 1955).

After these pioneering studies, although the failure of adult neurogenesis in the intestine has been reported by Gabella (Gabella 1984) probably due to application of inadequate counting methods, several studies confirmed the existence of a stem cell niche in the adult Auerbach's plexus (Geuna et al. 2010).

Notably, a series of papers from our research group have demonstrated that DNA synthesis, without cell division, may occur in neurons from the hypertrophic intestinal loops. This phenomenon was studied by autoradiography after ^3H -thymidine administration, while PCNA immunostaining demonstrated the presence of neosynthesized DNA in myenteric neurons (Corveti et al. 2001; Geuna et al.

1991; Giacobini et al. [n.d.](#)), and cytophotometry after Feulgen staining showed that DNA neosynthesis is associated with hyperdiploid DNA content (Geuna et al. [1991](#); Robecchi et al. [1988](#)). Yet, electrophoretic analysis of the total genomic DNA from Auerbach's ganglia belonging to hypertrophic loops revealed low-molecular-weight bands, thus revealing that DNA amplification is the required mechanism for DNA synthesis in adult myenteric neurons (Giacobini Robecchi et al. [1995](#)).

The occurrence of neurogenesis in the postnatal ANS has also been demonstrated in an experimental model of gut denervation induced by benzalkonium chloride (BAC) (Sakata et al. [1979](#); Sato et al. [1978](#)). Indeed, BAC administration induces the destruction of 90% of the Auerbach's plexus neurons followed by a significant increase (two- to fivefold) in neuronal density in the small ganglia located along the mesenteric nerves (Cracco and Filogamo [1993](#)). Interestingly, the location of mesenteric nerves corresponds to the migration pathway of the neural crest cells colonizing the gut during development. Since no neuronal mitoses were observed, it has been postulated that neuronal addition along mesenteric nerves might be due to the late differentiation of a niche of neural crest-derived undifferentiated elements that persist in adulthood. It has been proposed (Filogamo [1987](#); Varilek et al. [1991](#)) that this differentiation of neural crest-derived precursors can be triggered by factors originating from the intestine in the presence of degenerative and regenerative processes. Diffusible factors are able to induce neuronal differentiation; it was observed thanks to PC12 cell transplantation in the intestinal wall of BAC-treated animals contributing to the appearance of mature neurons (Filogamo and Cracco [1995](#)). Further evidence to support postnatal histogenesis in the ENS has also arisen from clinical studies demonstrating that inflammation induces an increase in the number of neurons in the myenteric ganglia (Andrew Kenneth [1953](#); Dockerty and Mayo [1955](#); Dockerty et al. [n.d.](#)). Sharkey and Parr (1996) confirmed that the myenteric neuron addition in adulthood derives from precursor cells of the myenteric ganglia able to proliferate late into adult neurons (Parr and Sharkey [1996](#)). The presence of a niche of reserve progenitor cells has also been reported in autonomic sympathetic ganglia and in the nodose ganglion (Celestino da Costa [1939](#); Picard and Chambost [1953](#)). In the latter case, it has been shown that neurogenesis can occur as a consequence of capsaicin treatment that induces a 50% reduction in the neuronal population followed by a rapid recovery of the pretreatment neuronal number. It has also been shown that a niche of neural progenitors that express nestin is present in nodose ganglion of adult rats and that capsaicin treatment is followed by the appearance of a significant population of BrdU/Ki-67-labeled cells, many of which differentiate into functional new neurons able to replace lost connections following capsaicin-induced damage (Chen et al. [2009](#); Gallaher et al. [2011](#); Ryu et al. [2010](#)).

The possibility that neurogenesis occurs in the adult intestine has also been confirmed by Hanani and colleagues (Hanani et al. [2003](#)), while Pardal (Pardal et al. [2007](#)) showed that glia-like stem cells sustain neurogenesis in the adult carotid body. A few years later, Joseph and Laranjeira (Joseph et al. [2011](#); Laranjeira et al. [2011](#)), investigating the stem cell niche in the ENS, confirmed the glial origin of enteric stem cells which can be activated by tissue dissociation or injury (Gershon

2011). Similar results were obtained by Uesaka and colleagues in mice (Uesaka et al. 2015), while, very recently, Woods et al. searched evidence for glial-derived neurogenesis in the human gut and showed the peculiar dual expression of glial and neuronal markers in rectal and perirectal neurons, thus suggesting that, like in rodents, Schwann cell-derived neurogenesis also supports the innervation of the human gastrointestinal tract (Woods et al. 2020).

In conclusion, while there is still some failure in confirming neurogenesis in some specific sites of the ANS such as sympathetic ganglia (Walters et al. 2020), the body of evidence produced so far suggests that the adult genesis of neurons is possible in the ANS and may be a way to promote neural repair and regeneration in pathological conditions and/or after injury. It can also be stated that adult neurogenesis in the ANS is sustained by glia-like stem cell niches of neural crest origin.

14 Conclusions

The ANS is a complex network comprising motor nerves responsible for essential visceral functions. It is well known that nerve structure can be frequently affected by clinical iatrogenic lesion that leads to organ impairment and disability. In this review the main factors influencing autonomic nerve regeneration and the most common clinical conditions have been considered, with a special focus on experimental models to study regeneration mechanisms. This body of evidence is expected to provide the basis for designing innovative strategies to prevent iatrogenic lesions during surgical treatments and to enhance the nerve regeneration process following an injury.

References

- Ambrosi F (1942) Sulle Alterazioni Istologiche Dei Gangli e Dei Nervi Del Tubo Digerente Nella Occlusione Intestinale. *Ricerche Nell'uomo e Sperimentali*. Arch Vecchi Anat Pat 5:215–240
- Andrew Kenneth S (1953) The myenteric plexus in ulcerative colitis. *Surg Gynae Obstets* 97: 335–343
- Antoniadis G, Kretschmer T, Pedro MT, König RW, Heinen CPG, Richter HP (2014) Iatrogene Nervenläsionen Prävalenz, Diagnostik Und Therapie. *Dtsch Arztebl Int* 111(16):273–279
- Archer DR, McLean WG (1988) Changes in fast axonally transported proteins in the regenerating rabbit vagus nerve. *Neurosci Lett* 87(1–2):151–156. [https://doi.org/10.1016/0304-3940\(88\)90161-9](https://doi.org/10.1016/0304-3940(88)90161-9)
- Awad M, Czer LS, Hou M, Golshani SS, Goltche M, De Robertis M, Kittleson M, Patel J, Azarbal B, Kransdorf E, Esmailian F, Trento A, Kobashigawa JA (2016) Early denervation and later reinnervation of the heart following cardiac transplantation: a review. *J Am Heart Assoc* 5(11): e004070. <https://doi.org/10.1161/JAHA.116.004070>. PMID: 27802930; PMCID: PMC5210323
- Belkind-Gerson J, Graeme-Cook F, Winter H (2006) Enteric nervous system disease and recovery, plasticity, and regeneration. *J Pediatr Gastroenterol Nutr* 42(4):343–350
- Belkind-Gerson J, Hotta R, Nagy N, Thomas AR, Graham H, Cheng L, Solorzano J, Nguyen D, Kamionek M, Dietrich J, Cherayil BJ, Goldstein AM (2015) Colitis induces enteric

- neurogenesis through a 5-HT4-dependent mechanism. *Inflamm Bowel Dis* 21(4):870–878. <https://doi.org/10.1097/MIB.0000000000000326>
- Bengel FM, Ueberfuhr P, Schiepel N, Nekolla SG, Reichart B, Schwaiger M (2001) Effect of sympathetic reinnervation on cardiac performance after heart transplantation. *N Engl J Med* 345 (10):731–738. <https://doi.org/10.1056/nejmoa010519>
- Benninghoff A (1951) Vermehrung Und Vergrößerung von Nervenzellen Bei Hypertrophie Des Innervationsgebietes. *Z Naturforsch – Sect B J Chem Sci* 6(1):38–41. <https://doi.org/10.1515/znb-1951-0109>
- Bergeijk V, Jeroen KH, Grothe C, Claus P (2006) Valproic acid promotes neurite outgrowth in PC12 cells independent from regulation of the survival of motoneuron protein. *Chem Biol Drug Des.* <https://doi.org/10.1111/j.1747-0285.2006.00369.x>
- Blennerhassett MG, Lourenssen SR (2021) Obligatory activation of SRC and JNK by GDNF for survival and axonal outgrowth of postnatal intestinal neurons. *Cell Mol Neurobiol.* <https://doi.org/10.1007/s10571-021-01048-9>
- Boyen V, Georg BT, Schulte N, Pflüger C, Spaniol U, Hartmann C, Steinkamp M (2011) Distribution of enteric glia and GDNF during gut inflammation. *BMC Gastroenterol* 11. <https://doi.org/10.1186/1471-230X-11-3>
- Boyle P, Ferlay J (2005) Mortality and survival in breast and colorectal cancer. *Nat Clin Pract Oncol* 2(9):424–425
- Braith RW, Wood CE, Limacher MC, Pollock ML, Lowenthal DT, Phillips MI, Staples ED (1992) Abnormal neuroendocrine responses during exercise in heart transplant recipients. *Circulation* 86(5):1453–1463. <https://doi.org/10.1161/01.CIR.86.5.1453>
- Buendia-Fuentes F, Almenar L, Ruiz C, Vercher JL, Sánchez-Lázaro I, Martínez-Dolz L, Navarro J, Bello P, Salvador A (2011) Sympathetic reinnervation 1 year after heart transplantation, assessed using iodine-123 metaiodobenzylguanidine imaging. *Transplant Proc* 43(6):2247–2248. <https://doi.org/10.1016/j.transproceed.2011.05.020>
- Cameron ML (1933) Regeneration of the peripheral vagus. *Q J Exp Physiol.* <https://doi.org/10.1113/expphysiol.1933.sp000597>
- Carter JT, Pisquiy JJ, Polmear M, Khalifa R, Gonzalez G (2020) A new classification of iatrogenic peripheral nerve injuries. *Biol Med* 12(3):1–3. <https://doi.org/10.35248/0974-8369.20.12.464>
- Celestino da Costa A (1939) Paraganglions et Sympathique. *Ann Endocrinol* 1:449–464
- Chen JH, Wei SZ, Chen J, Wang Q, Liu HL, Gao XH, Li GC, Yu WZ, Chen M, Luo HS (2009) Sensory denervation reduces visceral hypersensitivity in adult rats exposed to chronic unpredictable stress: evidences of neurogenic inflammation. *Dig Dis Sci* 54(9). <https://doi.org/10.1007/s10620-008-0575-5>
- Cooper T, Willman VL, Jellinek M, Rollins Hanlon C (1962) Heart autotransplantation: effect on myocardial catecholamine and histamine. *Science* 138(3536):40–41. <https://doi.org/10.1126/science.138.3536.40>
- Corvetti G, Fornaro M, Geuna S, Poncino A, Giacobini-Robecchi MG (2001) Unscheduled DNA synthesis in rat adult myenteric neurons: an immunohistochemical study. *Neuroreport* 12(10): 2165–2168. <https://doi.org/10.1097/00001756-200107200-00024>
- Cracco C, Filogamo G (1993) Mesenteric neurons in the adult rat are responsive to ileal treatment with benzalkonium chloride. *Int J Dev Neurosci* 11(1):49–61. [https://doi.org/10.1016/0736-5748\(93\)90034-B](https://doi.org/10.1016/0736-5748(93)90034-B)
- Dewulf K, Weyns V, Lelie B, Qasim H, Meersschaert J, Devos B (2020) Ectopic leiomyoma as a late complication of laparoscopic hysterectomy with power morcellation: a case report and review of the literature. *Acta Chir Belg* 120(5). <https://doi.org/10.1080/00015458.2019.1586396>
- Dockerty MB, Mayo CW (1955) The myenteric plexus in regional enteritis: a study of the number of ganglion cells in the ileum in 24 cases. *Surg Gynecol Obstet* 101:208–216
- Dockerty MB, Mayo CW, undefined 1955 (n.d.) The myenteric plexus in regional enteritis: a study of the number of ganglion cells in the ileum in 24 cases. *Surg Gynecol Obstet* 101. Europepmc.Org
- Doering LV, Dracup K, Moser DK, Czer LSC, Peter CT (1996) Hemodynamic adaptation to orthostatic stress after orthotopic heart transplantation. *Heart Lung: J Acute Crit Care* 25(5): 339–351. [https://doi.org/10.1016/S0147-9563\(96\)80076-8](https://doi.org/10.1016/S0147-9563(96)80076-8)

- Donadio V, Incensi A, Vacchiano V, Infante R, Magnani M, Liguori R (2019) The autonomic innervation of hairy skin in humans: an in vivo confocal study. *Sci Rep* 9(1). <https://doi.org/10.1038/s41598-019-53684-3>
- Filogamo G (1955) Iperplasia Ed Ipertrofia Delle Cellule Gangliari Del Plesso Sottomucoso (Di Meissner) Nel Cane, in Condizioni Sperimentali [Hyperplasia and hypertrophy of the Meissner's plexus ganglion cells in dogs, in experimental conditions]. *Riv Biol* 47:331–342
- Filogamo G (1987) Neuronal modulation by number and by volume: a review and critical analysis. In: *Model systems of development and aging of the nervous system*. Springer US, pp 85–100
- Filogamo G, Cracco C (1995) Models of neuronal plasticity and repair in the enteric nervous system: a review. *Ital J Anat Embryol* 100(Suppl):185–195
- Filogamo G, Vigliani F (1953) Le Cellules Du Plexus Myentérique (d'Auerbach) Dans La Sténose Expérimentale de l'intestin. *CR Assoc Anat* 78:683–692
- Filogamo G, Vigliani F (1954) Ricerche Sperimentale Sulla Correlazione Tra Estensione Del Territorio Di Innervazione e Grandezza e Numero Delle Cellule Gangliari Del Plesso Mienterico (Di Auerbach), Nel Cane. Società it. edited by Mondino in Pavia
- Flecknell P (2002) Replacement, reduction and refinement. *ALTEX: Alternativen Zu Tierexperimenten*
- Fregnan F, Ciglieri E, Tos P, Crosio A, Ciardelli G, Ruini F, Tonda-Turo C, Geuna S, Raimondo S (2016) Chitosan crosslinked flat scaffolds for peripheral nerve regeneration. *Biomed Mater* (Bristol). <https://doi.org/10.1088/1748-6041/11/4/045010>
- Fu SY, Gordon T (1995) Contributing factors to poor functional recovery after delayed nerve repair: prolonged denervation. *J Neurosci*. <https://doi.org/10.1523/jneurosci.15-05-03886.1995>
- Fujii S (2008) Anatomic identification of nerve-sparing radical hysterectomy: a step-by-step procedure. *Gynecol Oncol* 111(2 Suppl):S33–S41. <https://doi.org/10.1016/j.ygyno.2008.07.026>
- Furness JB (2006) The organisation of the autonomic nervous system: peripheral connections. *Auton Neurosci: Basic Clin* 130(1–2):1–5
- Furness JB (2008) The enteric nervous system: normal functions and enteric neuropathies. *Neurogastroenterol Motil* 20(Suppl 1):32–38
- Gabella G (1984) Size of neurons and glial cells in the intramural ganglia of the hypertrophic intestine of the Guinea-pig. *J Neurocytol* 13(1):73–84. <https://doi.org/10.1007/BF01148319>
- Gabella G (1987) The number of neurons in the small intestine of mice, Guinea-pigs and sheep. *Neuroscience* 22(2):737–752. [https://doi.org/10.1016/0306-4522\(87\)90369-1](https://doi.org/10.1016/0306-4522(87)90369-1)
- Gallaher ZR, Ryu V, Larios RM, Sprunger LK, Czaja K (2011) Neural proliferation and restoration of neurochemical phenotypes and compromised functions following capsaicin-induced neuronal damage in the Nodose ganglion of the adult rat. *Front Neurosci* (FEB). <https://doi.org/10.3389/fnins.2011.00012>
- Gaudet AD, Popovich PG, Ramer MS (2011) Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. *J Neuroinflammation* 8(1):1–13
- Genton L, Kudsk KA (2003) Interactions between the enteric nervous system and the immune system: role of neuropeptides and nutrition. *Am J Surg* 186(3):253–258
- Gershon MD (2011) Behind an enteric neuron there may lie a glial cell. *J Clin Investig* 121(9):3386–3389
- Geuna S, Poncino A, Giacobini Robecchi MG (1991) DNA content revealed by cytophotometry in neurons: variability related to neuroplasticity. *Adv Exp Med Biol* 296:13–19
- Geuna S, Tos P, Battiston B (2009) Essays on peripheral nerve repair and regeneration. Elsevier
- Geuna S, Fornaro M, Raimondo S, Giacobini-Robecchi MG (2010) Plasticity and regeneration in the peripheral nervous system. *Ital J Anat Embryol* 115(1–2):91–4. PMID: 21072996
- Geuna S, Perroteau I, Tos PL, Battiston B (2013) Tissue engineering of the peripheral nerve – biomaterials and physical therapy, vol 109. Elsevier Science
- Geuna S, Muratori L, Fregnan F, Manfredi M, Bertolo R, Porpiglia F (2018) Strategies to improve nerve regeneration after radical prostatectomy: a narrative review. *Minerva Urol Nefrol* 70(6):546–558

- Giacobini RMG, ... M. Cannas-International journal of, undefined 1985 (n.d.) Increase in the number and volume of myenteric neurons in the adult rat. Ncbi.Nlm.Nih.Gov
- Greene LA, Tischler AS (1976) Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proc Natl Acad Sci U S A* 73(7):2424–8. <https://doi.org/10.1073/pnas.73.7.2424>. PMID: 1065897; PMCID: PMC430592
- Grinsell D, Keating CP (2014) Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. *BioMed Res Int* 2014:1
- Grupper A, Gewirtz H, Kushwaha S (2018) Reinnervation post-heart transplantation. *Eur Heart J* 39(20):1799–1806
- Hanani M, Ledder O, Yutkin V, Abu-Dalu R, Huang TY, Härtig W, Vannucchi MG, Faussone-Pellegrini MS (2003) Regeneration of myenteric plexus in the mouse colon after experimental denervation with benzalkonium chloride. *J Comp Neurol* 462(3):315–327. <https://doi.org/10.1002/cne.10721>
- Hannan JL, Powers SA, Wang VM, Castiglione F, Hedlund P, Bivalacqua TJ (2017) Impaired contraction and decreased detrusor innervation in a female rat model of pelvic neuropraxia. *Int Urogynecol J*. <https://doi.org/10.1007/s00192-016-3223-1>
- Hayman MA, Nativi JN, Stehlik J, McDaniel J, Fjeldstad AS, Ives SJ, Walter Wray D, Bader F, Gilbert EM, Richardson RS (2010) Understanding exercise-induced hyperemia: central and peripheral hemodynamic responses to passive limb movement in heart transplant recipients. *Am J Physiol Heart Circ Physiol* 299(5):H1653. <https://doi.org/10.1152/ajpheart.00580.2010>
- Hu H, Spencer NJ (2018) Enteric nervous system structure and neurochemistry related to function and neuropathology. In: *Physiology of the gastrointestinal tract*, 6th edn. Academic
- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ (2008a) Cancer statistics, 2008. *CA Cancer J Clin* 58(2):71–96. <https://doi.org/10.3322/ca.2007.0010>
- Jemal A, Thun MJ, Ries LAG, Howe HL, Weir HK, Center MM, Ward E, Wu XC, Ehemann C, Anderson R, Ajani UA, Kohler B, Edwards BK (2008b) Annual report to the nation on the status of cancer, 1975–2005, featuring trends in lung cancer, tobacco use, and tobacco control. *J Natl Cancer Inst* 100(23):1672–1694. <https://doi.org/10.1093/jnci/djn389>
- Jiman AA, Ratze DC, Welle EJ, Patel PR, Richie JM, Bottorff EC, Seymour JP, Chestek CA, Bruns TM (2020) Multi-channel intraneural vagus nerve recordings with a novel high-density carbon fiber microelectrode array. *Sci Rep* 10(1). <https://doi.org/10.1038/s41598-020-72512-7>
- Jonscher R, Belkind-Gerson J (2019) Concise review: cellular and molecular mechanisms of postnatal injury-induced enteric neurogenesis. *Stem Cells* 37(9):1136–1143
- Joseph NM, He S, Quintana E, Kim YG, Núñez G, Morrison SJ (2011) Enteric glia are multipotent in culture but primarily form glia in the adult rodent gut. *J Clin Invest* 121(9):3398–3411. <https://doi.org/10.1172/JCI58186>
- Kaye DM, Esler M, Kingwell B, McPherson G, Esmore D, Jennings G (1993) Functional and neurochemical evidence for partial cardiac sympathetic reinnervation after cardiac transplantation in humans. *Circulation* 88(3):1110–1118. <https://doi.org/10.1161/01.CIR.88.3.1110>
- Kim HA, Mindos T, Parkinson DB (2013) Plastic fantastic: Schwann cells and repair of the peripheral nervous system. *Stem Cells Transl Med* 2(8):553–557. <https://doi.org/10.5966/sctm.2013-0011>
- Krenz NR, Weaver LC (1998) Changes in the morphology of sympathetic preganglionic neurons parallel the development of autonomic dysreflexia after spinal cord injury in rats. *Neurosci Lett* 243(1–3):61–64. [https://doi.org/10.1016/S0304-3940\(98\)00101-3](https://doi.org/10.1016/S0304-3940(98)00101-3)
- Kreutzberg GW (2009) Reaction of the neuronal cell body to axonal damage. In: *The axon: structure, function and pathophysiology*. Oxford University Press
- Lake JJ, Heuckeroth RO (2013) Enteric nervous system development: migration, differentiation, and disease. *Am J Physiol Gastrointest Liver Physiol* 305(1). <https://doi.org/10.1152/AJPGL.00452.2012>
- Laranjeira C, Sandgren K, Kessaris N, Richardson W, Potocnik A, Berghe PV, Pachnis V (2011) Glial cells in the mouse enteric nervous system can undergo neurogenesis in response to injury. *J Clin Invest* 121(9):3412–3424. <https://doi.org/10.1172/JCI58200>

- Lin YF, Wang JY, Denq JC, Lin SH (2003) Midodrine improves chronic hypotension in hemodialysis patients. *Am J Med Sci* 325(5):256–261. <https://doi.org/10.1097/00000441-200305000-00002>
- Lovric SS, Avbelj V, Trobec R, Zorman D, Rakovec P, Hojker S, Gersak B, Milcinski M (2004) Sympathetic reinnervation after heart transplantation, assessed by iodine-123 meta-iodobenzylguanidine imaging, and heart rate variability. *Eur J Cardiothorac Surg* 26(4):736–741. <https://doi.org/10.1016/j.ejcts.2004.07.025>
- Mackinnon SE (1989) New directions in peripheral nerve surgery. *Ann Plast Surg* 22(3):257–273
- Magrina J, Yang J, Yi J, Wasson M (2021) Nerve-sparing in gynecologic surgery: a perspective. *J Minim Invasive Gynecol* 28(3):475–480. <https://doi.org/10.1016/j.jmig.2020.07.009>
- McCorry LK (2007) Physiology of the autonomic nervous system. *Am J Pharm Educ* 71(4). <https://doi.org/10.5688/aj710478>
- McLean WG (1985) Axonal transport of actin and regeneration rate in non-myelinated sensory nerve fibres. *Brain Res* 333(2):255–260. [https://doi.org/10.1016/0006-8993\(85\)91579-3](https://doi.org/10.1016/0006-8993(85)91579-3)
- McRae BR, Shew M, Aaron GP, Bijangi-Vishehsaraei K, Halum SL (2012) A rapid, novel model of culturing cranial nerve X-derived motoneurons for screening trophic factor outgrowth response. *Neurol Res*. <https://doi.org/10.1179/1743132812Y.0000000046>
- Menorca RMG, Fussell TS, Elfär JC (2013) Nerve physiology. Mechanisms of injury and recovery. *Hand Clin* 29(3):317–330
- Miura M (1913) Aus Den Notizien Meiner Physiologischen Und Patologischen Forschung. In: *Muscularis Mucosae et Muscularis Propria Gastrointestinalis*. Nakōdo-Verlag, pp 193–215
- Moore AE, Zhang J, Stringer MD (2012) Iatrogenic nerve injury in a national no-fault compensation scheme: an observational cohort study. *Int J Clin Pract* 66(4):409–416. <https://doi.org/10.1111/j.1742-1241.2011.02869.x>
- Muratori L, Fregnan F, Ronchi G, Haastert-Talini K, Metzen J, Bertolo R, Porpiglia F, Geuna S (2019) New basic insights on the potential of a chitosan-based medical device for improving functional recovery after radical prostatectomy. *BJU Int* 124(6):1063–1076. <https://doi.org/10.1111/bju.14834>
- Namkoong C, Toshinai K, Zave Waise TM, Sakoda H, Sasaki K, Ueta Y, Kim MS, Minamino N, Nakazato M (2017) NERP-2 regulates gastric acid secretion and gastric emptying via the orexin pathway. *Biochem Biophys Res Commun*. <https://doi.org/10.1016/j.bbrc.2017.02.064>
- Navarro X (2016) “Functional evaluation of peripheral nerve regeneration and target reinnervation in animal models: a critical overview” edited by J. Foxe. *Eur J Neurosci* 43(3):271–286. <https://doi.org/10.1111/ejn.13033>
- Navarro X, Vivó M, Valero-Cabré A (2007) Neural plasticity after peripheral nerve injury and regeneration. *Prog Neurobiol* 82(4):163–201
- Norvell JE, Lower RR (1973) Degeneration and regeneration of the nerves of the heart after transplantation. *Transplantation* 15(3):337–344
- Odom MR, Powers SA, Moomaw MA, Pak ES, Ashcraft KA, Koontz BF, Hannan JL (2018) 017 differential effects on MPG neurons and penile vasculature after conformal prostate radiation in rats. *J Sex Med*. <https://doi.org/10.1016/j.jsxm.2017.11.036>
- Pardal R, Ortega-Sáenz P, Durán R, López-Barneo J (2007) Glia-like stem cells sustain physiologic neurogenesis in the adult mammalian carotid body. *Cell* 131(2):364–377. <https://doi.org/10.1016/j.cell.2007.07.043>
- Parr EJ, Sharkey KA (1996) Immunohistochemically-defined subtypes of neurons in the inferior mesenteric ganglion of the Guinea-pig. *J Auton Nerv Syst* 59(3):140–150. [https://doi.org/10.1016/0165-1838\(96\)00017-3](https://doi.org/10.1016/0165-1838(96)00017-3)
- Parry DS, Foulsham L, Jenkins G, Wharton J, Marron K, Banner N, Yacoub M (1997) Incidence and functional significance of sympathetic reinnervation after cardiac transplantation. In: *Transplantation proceedings*, vol 29. Elsevier, pp 569–570
- Parsaik AK, Singh B, Altayar O, Mascarenhas SS, Singh SK, Erwin PJ, Hassan Murad M (2013) Midodrine for orthostatic hypotension: a systematic review and meta-analysis of clinical trials. *J Gen Intern Med* 28(11):1496–1503
- Patel VR, Samavedi S, Bates AS, Kumar A, Coelho R, Rocco B, Palmer K (2015) Dehydrated human amnion/chorion membrane allograft nerve wrap around the prostatic neurovascular

- bundle accelerates early return to continence and potency following robot-assisted radical prostatectomy: propensity score-matched analysis. *Eur Urol* 67(6):977–980. <https://doi.org/10.1016/j.eururo.2015.01.012>
- Picard D, Chambost M (1953) Les Cellules de Remplacement Dans Le Système Nerveux Vegetatif et Les Paraganglions: Observations Sur Les Formations Nerveuses Intraspinales. *Arch Anat Microsc Morphol* 42:86–101
- Porpiglia F, Bertolo R, Fiori C, Manfredi M, De Cillis S, Geuna S (2018) Chitosan membranes applied on the prostatic neurovascular bundles after nerve-sparing robot-assisted radical prostatectomy: a phase II study. *BJU Int* 121(3):472–478. <https://doi.org/10.1111/bju.13959>
- Querleu D, Paul Morrow C (2008) Classification of radical hysterectomy. *Lancet Oncol* 9(3): 297–303
- Querleu D, Cibula D, Abu-Rustum NR (2017) 2017 update on the Querleu-Morrow classification of radical hysterectomy. *Ann Surg Oncol* 24(11):3406–3412. <https://doi.org/10.1245/s10434-017-6031-z>. Epub 2017 Aug 7. PMID: 28785898; PMCID: PMC6093205
- Quinlan DM, Nelson RJ, Partin AW, Mostwin JL, Walsh PC (1989) The rat as a model for the study of penile erection. *J Urol* 141(3 I):656–661. [https://doi.org/10.1016/S0022-5347\(17\)40926-8](https://doi.org/10.1016/S0022-5347(17)40926-8)
- Randolph JT, Pak ES, Koontz BF, Hannan JL (2020) Ex vivo radiation leads to opposing neurite growth in whole ganglia vs dissociated cultured pelvic neurons. *J Sex Med*. <https://doi.org/10.1016/j.jsxm.2020.04.385>
- Robecchi MGG, Poncino A, Geuna S, Giacometti S, Filogamo G (1988) DNA content in neurons of Auerbach's plexus under experimental conditions in adult rats. *Int J Dev Neurosci* 6(2):109–115. [https://doi.org/10.1016/0736-5748\(88\)90034-2](https://doi.org/10.1016/0736-5748(88)90034-2)
- Robecchi G, Maria G, Borriore P, Canavese M, Guena S, Paraninfo A, Poncino A, Silencio L (1995) DNA neosynthesis in Auerbach plexus ganglia isolated from the rat hypertrophic gut: an electrophoretic analysis. *Int J Dev Neurosci* 13(6):635–637. [https://doi.org/10.1016/0736-5748\(95\)00035-F](https://doi.org/10.1016/0736-5748(95)00035-F)
- Ryu V, Gallaher Z, Czaja K (2010) Plasticity of nodose ganglion neurons after capsaicin- and vagotomy-induced nerve damage in adult rats. *Neuroscience* 167(4). <https://doi.org/10.1016/j.neuroscience.2010.02.049>
- Sakata K, Kunieda T, Furuta T, Sato A (1979) Selective destruction of intestinal nervous elements by local application of benzalkonium solution in the rat. *Experientia* 35(12):1611–1613. <https://doi.org/10.1007/BF01953222>
- Saper CB (2002) The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. *Annu Rev Neurosci* 25:433–469
- Sato A, Yamamoto M, Imamura K, Kashiki Y, Kunieda T, Sakata K (1978) Pathophysiology of aganglionic colon and anorectum: an experimental study on aganglionosis produced by a new method in the rat. *J Pediatr Surg* 13(4):399–405. [https://doi.org/10.1016/S0022-3468\(78\)80464-3](https://doi.org/10.1016/S0022-3468(78)80464-3)
- Schrage WG, Eisenach JH, Dinunno FA, Roberts SK, Johnson CP, Sandroni P, Low PA, Joyner MJ (2004) Effects of midodrine on exercise-induced hypotension and blood pressure recovery in autonomic failure. *J Appl Physiol* 97(5):1978–1984. <https://doi.org/10.1152/jappphysiol.00547.2004>
- Schuster A, Klotz M, Schwab T, Di Liddo R, Bertalot T, Schrenk S, Martin M, Nguyen TD, Nguyen TNQ, Gries M, Faßbender K, Conconi MT, Parnigotto PP, Schäfer KH (2014) Maintenance of the enteric stem cell niche by bacterial lipopolysaccharides? Evidence and perspectives. *J Cell Mol Med* 18(7):1429–1443. <https://doi.org/10.1111/jcmm.12292>
- Seddon HJ (1942) A classification of nerve injuries. *Br Med J* 2(4260):237. <https://doi.org/10.1136/bmj.2.4260.237>
- Sekiyama K, Ando Y, Taga A, Kozono Y, Higuchi T, Fujii S (2020) Laparoscopic technique for step-by-step nerve-sparing Okabayashi radical hysterectomy. *Int J Gynecol Cancer* 30(2): 276–277. <https://doi.org/10.1136/ijgc-2019-000726>
- Sharifi AM, Mousavi SH, Farhadi M, Larijani B (2007) Study of high glucose-induced apoptosis in PC12 cells: role of Bax protein. *J Pharmacol Sci*. <https://doi.org/10.1254/jphs.FP0070258>
- Stinson EB, Griep RB, Schroeder JS, Dong E, Shumway NE (1972) Hemodynamic observations one and two years after cardiac transplantation in man. *Circulation* 45(6):1183–1194. <https://doi.org/10.1161/01.CIR.45.6.1183>

- Storr MA, Keenan CM, Emmerdinger D, Zhang H, Yüce B, Sibaev A, Massa F, Buckley NE, Lutz B, Göke B, Brand S, Patel KD, Sharkey KA (2008) Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB1 and CB2 receptors. *J Mol Med* 86(8):925–936. <https://doi.org/10.1007/s00109-008-0359-6>
- Sulaiman W, Gordon T (2013) Neurobiology of peripheral nerve injury, regeneration, and functional recovery: from bench top research to bedside application. *Ochsner J* 13:100
- Sunderland S (1951) A classification of peripheral nerve injuries producing loss of function. *Brain* 74(4):491–516. <https://doi.org/10.1093/brain/74.4.491>
- Swenson RS (2006) Review of clinical and functional neuroscience, Chapter 9. In: Educational review manual in neurology. Castle Connolly Graduate Medical Publishing
- Überfuhr P, Frey AW, Ziegler S, Reichart B, Schwaiger M (2000a) Sympathetic reinnervation of sinus node and left ventricle after heart transplantation in humans: regional differences assessed by heart rate variability and positron emission tomography. *J Heart Lung Transplant* 19(4):317–323. [https://doi.org/10.1016/S1053-2498\(00\)00060-7](https://doi.org/10.1016/S1053-2498(00)00060-7)
- Überfuhr P, Ziegler S, Schwaiblmair M, Reichart B, Schwaiger M (2000b) Incomplete sympathetic reinnervation of the orthotopically transplanted human heart: observation up to 13 years after heart transplantation. *Eur J Cardiothorac Surg* 17(2):161–168. [https://doi.org/10.1016/S1010-7940\(99\)00367-X](https://doi.org/10.1016/S1010-7940(99)00367-X)
- Uesaka T, Nagashimada M, Enomoto H (2015) Neuronal differentiation in Schwann cell lineage underlies postnatal neurogenesis in the enteric nervous system. *J Neurosci* 35(27):9879–9888. <https://doi.org/10.1523/JNEUROSCI.1239-15.2015>
- Varilek GW, Weinstock JV, Williams TH, Jew J (1991) Alterations of the intestinal innervation in mice infected with schistosoma mansoni. *J Parasitol* 77(3):472–478. <https://doi.org/10.2307/3283138>
- Verleye L, Vergote I, Reed N, Ottevanger PB (2009) Quality assurance for radical hysterectomy for cervical cancer: the view of the European Organization for Research and Treatment of Cancer – Gynecological Cancer Group (EORTC-GCG). *Ann Oncol* 20(10):1631–1638. <https://doi.org/10.1093/ANNONC/MDP196>
- Verma N, Singh AP, Rao CV (2019) Antioxidant mediated ulcer healing potential of hyperin isolated from flowers of rhododendron arboreum in experimental rat. *Int J Pharmaceut Sci Res* 10:1524
- Walsh KT, Zemper AE (2019) The enteric nervous system for epithelial researchers: basic anatomy, techniques, and interactions with the epithelium. CMGH
- Walters KM, Boucher M, Boucher GG, Opsahl AC, Mouton PR, Liu CN, Ritenour CR, Kawabe TT, Pyski HN, Soms CJ (2020) No evidence of neurogenesis in adult rat sympathetic ganglia following guanethidine-induced neuronal loss. *Toxicol Pathol* 48(1):228–237. <https://doi.org/10.1177/0192623319843052>
- Wang ML, Rivlin M, Graham JG, Beredjikian PK (2019) Peripheral nerve injury, scarring, and recovery. *Connect Tissue Res*. <https://doi.org/10.1080/03008207.2018.1489381>
- Waxenbaum JA, Varacallo M (2019) Anatomy, autonomic nervous system. StatPearls Publishing
- Wilson RF, Christensen BV, Olivari MT, Simon A, White CW, Laxson DD (1991) Evidence for structural sympathetic reinnervation after orthotopic cardiac transplantation in humans. *Circulation* 83(4):1210–1220. <https://doi.org/10.1161/01.CIR.83.4.1210>
- Wilson RF, Johnson TH, Haidet GC, Kubo SH, Mianuelli M (2000) Sympathetic reinnervation of the sinus node and exercise hemodynamics after cardiac transplantation. *Circulation* 101(23):2727–2733. <https://doi.org/10.1161/01.CIR.101.23.2727>
- Woods C, Kapur RP, Bischoff A, Lovell M, Arnold M, Peña A, Flockton A, Sharkey KA, Belkind-Gerson J (2020) Neurons populating the rectal extrinsic nerves in humans express neuronal and Schwann cell markers. *Neurogastroenterol Motil* 33:14074. <https://doi.org/10.1111/nmo.14074>
- Wu HF, Zeltner N (2020) Efficient differentiation of postganglionic sympathetic neurons using human pluripotent stem cells under feeder-free and chemically defined culture conditions. *J Vis Exp* 2020(159):1–14. <https://doi.org/10.3791/60843>
- Yan J-G, Shen F-Y, Thayer J, Yan Y, LoGiudice J, Matloub H, Sanger J, Zhang L-L, Havlik R (2017) Repair of the musculocutaneous nerve using the vagus nerve as donor by helicoid end-to-side technique: an experimental study in rats. *J Neurosci Res* 95(12):2493–2499. <https://doi.org/10.1002/jnr.24074>

Part II

Models and Evaluation of Peripheral Nerve Regeneration

Appropriate Animal Models for Translational Nerve Research

Kirsten Haastert-Talini

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Abstract

This chapter will focus on aspects to be considered when evaluating the properties of novel materials and constructs for bioartificial and tissue-engineered nerve graft repair in preclinical research. The potential for propagation of a novel nerve guide into a medical product for clinical use is significantly increased after its regeneration-supporting properties have been proven in specific and comprehensive in vitro and in vivo studies. The predictability toward clinical outcome is of outmost importance for translational research. This is why experimental

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investigation of novel nerve grafts should deliver results that have been comprehensively evaluated in appropriate models. Therefore, thoughtful study design is a main concern as is also publishing of conclusive results presenting novel developments eventually holding great promise and deserving the enterprise to be processed toward a clinical product. Nevertheless, comprehensive analysis will also reveal developments that are clearly less promising for various reasons, and also these results should be published in order to speed up straightforward modification of the attempt or to avoid others from wasting time, money, and human and animal resources. In the following paragraphs, considerations on the most appropriate, mainly in vivo, models for translational research in peripheral nerve repair will be presented. During this the reader will also be referred to some recently published excellent review articles that have already substantially contributed to the discussion and deserve deeper recognition from the interested reader. As in all other fields of biomedical research, careful study design should also be considered as a valuable tool to speed up the process for novel developments to achieve clinical approval for the eventual benefit of the patients.

1 Introduction

Sir Sydney Sunderland (1910–1993), who is often referred to as the “father of modern nerve surgery” (Siqueira and Kline 2018), is cited with the words: “The central objective of nerve repair is to assist regenerating axons to re-establish useful functional connections with the periphery” (Brushart 2011a; Siqueira and Martins 2017).

From this sentence, the intention for clinical surgery on injured peripheral nerves, especially for their release from compression or reconstruction after transection injury, is very clear. It is the need to surgically prepare an optimized environment (the optimally reconstructed damaged nerve) that will allow for sufficient axonal regeneration and their appropriate reconnection to peripheral targets for recovery of motor and sensory functions. Over the last decades, microsurgical techniques have been brought up to a level for which it is hard to imagine that future developments could further increase functional outcome after peripheral nerve repair surgery based on its own (Geuna et al. 2013). Therefore, basic research is needed to give ideas on how benefit could be achieved for patients suffering impairments after peripheral nerve damage, possibly for all their life (Grinsell and Keating 2014).

Also, the general objectives for neurobiological basic research with regard to peripheral nerve regeneration processes can again easily be derived from Sir Sunderland’s directive. Detailed knowledge about the molecular, cellular, and functional responses within the peripheral nerves’ proximal and distal segments, after injury and during regeneration, is crucial for tackling those events that remain not adequately addressed with current strategies for nerve repair. Indeed, basic research is continuously increasing our knowledge (1) about key events orchestrating peripheral nerve regeneration, (2) about basic mechanisms and pathways to be activated or

stopped for axonal regeneration and correct rewiring to peripheral targets, (3) about cellular key players and how lineage reprogramming is making them to the same, and, last but not least, (4) about the timing in which crucial events have to follow after another to allow functional recovery also after longer periods of denervation. We also learn more about how other systemic conditions, e.g., diabetes, or chemotherapy against various cancer types, could induce peripheral neuropathies and could therefore possibly further impact regeneration after peripheral nerve damage.

Experimental work on the tissue engineering of peripheral nerves is, however, primarily undertaken for developing a substitute for autologous nerve transplantation (Gu et al. 2014) or an established alternative, the so-called muscle-in-vein graft (Schiefer et al. 2015). This will be of benefit for patients needing reconstruction of multiple or largely extended peripheral nerve defects and for whom availability of autologous material is limited. Already in the last two decades of the nineteenth century, investigative surgeons have evaluated diverse nerve tubulization strategies in chicken, rabbits, and dogs for bridging a gap of up to 3 cm in length between transected peripheral nerve ends with tubular implants (autologous bone or vessel) (Fields et al. 1989). In these studies, successful regeneration was verified mainly by means of histomorphological evaluation (detection of regenerated axons in the distal nerve segment) and, only in some cases, by analyzing the electrodiagnostical properties of regenerated axons (Fields et al. 1989). With large-scale adoption of the surgical microscope, peripheral nerve repair and regeneration studies were increasingly often performed in rats. The rat sciatic nerve model, to this day, remains the most commonly employed animal model in this area (Angius et al. 2012; Geuna 2015).

In recent years, several leading experts in the field have discussed and reviewed the prevalence as well as the pros and cons of animal models used in preclinical evaluation of novel bioartificial and tissue-engineered nerve grafts. While this chapter will summarize the leading thoughts, but also the most critical ones, in order to serve the reader in appropriately designing future peripheral nerve regeneration studies with strong translational impact, Table 1 lists a number of references that the author finds worth featuring in this context.

2 Translational Aspects Should Already Be Considered for In Vitro Studies

The biomaterial field is frequently bringing up novel developments, and the results of their *in vitro* evaluation are published in peer-reviewed journals with varying impact factors. Their real impact for future development of novel nerve implants is, however, crucially depending not only on the publication of the data but also on the *in vitro* models used. Pure biocompatibility studies conducted with cell types that are not specific for (1) the peripheral nervous system, or (2) the area of neural regeneration in focus, or (3) that are solely performed on a single cell line, even if derived from peripheral nervous system cells, may not be convincing enough. Only convincing, comprehensive *in vitro* data can justify and foster preclinical *in vivo*

Table 1 Helpful review articles that have been published in recent years and should be considered for designing meaningful translational preclinical studies

Study type mainly referred to	Main topic	Reference
In vitro	Cell culture including organotypic cultures	Geuna et al. 2016
	Cell culture including lab-on-a-chip designs	Mobini et al. 2019
In vivo	Prevalence of preclinical models in use	Angius et al. 2012
	The rat sciatic nerve injury and repair model	Geuna 2015
	Utilization of specific rat nerves for studying different aspects of regeneration	Gordon and Borschel 2017
	Specific consideration of denervation times and observation periods	Hoke 2006
	Effective translatability of rat studies for clinical innovation	Kaplan et al. 2015
	Overview of functional assessments in use and outcome to be expected	Brushart 2011b, c
	The 3Rs of peripheral nerve regeneration and state-of-the-art evaluation of target reinnervation	Navarro 2016

testing followed by clinical evaluation of a new approach or tissue-engineered nerve graft development. With no doubt, in vitro studies utilizing cell lines indeed reveal important information for the still ongoing discovery of mechanisms that are crucial for the regeneration process. For substantial evidence that novel biomaterials or scaffolds represent interesting candidates for the development of tissue-engineered nerve grafts, however, in vitro biocompatibility studies need to be complemented by evaluation of the behavior of primary nerve cells, e.g., dorsal root ganglion neurons, spinal motor neurons, or Schwann cells, on these substrates (Geuna et al. [2016](#)). From an ethical point of view, in vitro studies using cutting-edge techniques are clearly warranted to reduce the number of animals devoted to early stage in vivo experiments (Geuna et al. [2016](#)), and recent progress on the sector of lab-on-a-chip devices will further broaden the possibilities for this (Mobini et al. [2019](#)). Refined and sophisticated in vitro models are already available today, e.g., those evaluating neurite outgrowth (1) from dorsal root ganglia into a three-dimensional matrix (Bozkurt et al. [2009](#); Grasman et al. [2018](#)) or (2) even from spinal cord slices into peripheral nerve segments or nerve guide materials (Vyas et al. [2010](#); Siddique et al. [2014](#)). These models, however, are yet difficult to establish and to propagate. Furthermore, translational research will always request well-justified preclinical in vivo evaluation. Preclinical in vivo evaluation of a novel graft type is legitimized, whenever preceding in vitro tests have demonstrated (1) biocompatibility with primary peripheral nervous system cells, or organotypic cultures from dorsal root ganglia, or spinal cord preparations, and (2) an evidently stimulated neurite outgrowth from primary sensory or motor neurons (Geuna et al. [2016](#)).

When preclinical *in vivo* testing of a novel nerve graft type is finally considered, the choice for the most appropriate animal model depends not only on the working hypothesis but also on local capacities or the availability of collaboration partners with strong experience in the model of interest. In any case, researchers need to consider that the results of their study will only be considered translational if objective and reliable functional testing is included in their preclinical evaluation (Navarro 2016).

3 The Obstacles in Making Preclinical In Vivo Studies Strong from a Translational Point of View

Appropriate *in vivo* models for evaluating the potential of innovative alternative nerve graft constructs have to mimic specific processes that do also occur during peripheral nerve regeneration in humans (Angius et al. 2012). After microsurgery has become routine in peripheral nerve repair, the rat sciatic nerve model of transection and repair has become the most commonly used model (Tos et al. 2009; Siemionow et al. 2010; Angius et al. 2012; Geuna 2015; Gordon and Borschel 2017). The reason for this clearly depends on economy, since it is easy to enroll larger experimental groups or to increase the number of groups investigated.

One remarkable disadvantage of rodent models is, though, the increased speed of axonal regeneration in comparison to humans. A second disadvantage is that in opposite to the clinical need in human patients in need of long nerve gap repair (5–30 cm) (Kaplan et al. 2015), only considerably shorter nerve gaps can be bridged (Angius et al. 2012; Kaplan et al. 2015) and that therefore mainly short denervation times are investigated (Hoke 2006; Kaplan et al. 2015). Also it has been perceived critically that most models use acute experimental repair approaches instead of substantially delayed secondary repair procedures. Delayed secondary repair would result in prolonged times for which (1) axons remain in a chronic axotomy condition, irrespective of their formation of axonal sprouts, and (2) Schwann cells in the distal nerve remain chronically denervated as long as no axonal sprouts reach them (Hoke 2006). Essentially only the research group of Tessa Gordon has repetitively analyzed the processes and modifications occurring in chronic (several months) denervation models. They established a common peroneal-tibial nerve cross-reinnervation paradigm, which enabled the independent assessment of events related to chronic axotomy and chronic Schwann cell denervation (Gordon and Borschel 2017). These models fulfill the request for a postponed nerve reconstruction in small animal models that more appropriately mimic the conditions at the lesion site and inside the distal nerve segment in long-gap repair in human patients (Hoke 2006).

Until today only few studies, besides the considerable number undertaken by Tessa Gordon and her colleagues, have adopted the experimental delayed repair paradigm for testing novel nerve graft developments in rat models. We have done so in two studies (Stenberg et al. 2017; Stossel et al. 2018) but only used a moderate delay time of 45 days after rat sciatic nerve transection injury. We considered the

period of 45 days to be sufficient for inducing biological effects related to a reduced regeneration capacity and to a reduced capacity of Schwann cells to support the regeneration process (Stenberg et al. 2017). For preventing spontaneous regeneration after primary nerve transection injury, we sutured the proximal and distal nerve ends to themselves, which also left a large enough gap between the separated nerve ends. With this procedure, however, it was not possible to include the classic autologous nerve graft (gold standard) control group in our experiments (Stossel et al. 2018). In this specific control group in acute repair studies, we would transect the distal nerve a second time at the distance of choice before suturing the reversed and 90° shifted nerve segment as a bridge between the proximal and the distal nerve ends. In the delayed repair studies, we found the separated nerve ends retracted after 45 days, and harvesting an autologous nerve graft from the same nerve for tension-free nerve repair was not possible anymore. Therefore, one would either need permission from the animal welfare committee to use the contralateral nerve as a substitute or go for an allograft repair with other potentially modifying consequences for the regeneration process (e.g., due to necessary immunosuppression, not in focus of this chapter).

The above example already demonstrates the difficulty in setting up small animal studies with a high translational impact. But criticism of currently used animal models, particularly broadly used rat models, in evaluating novel tissue-engineered nerve grafts goes further and therefore needs to be considered here as well.

The first additional criticism relates to the site of injury that is investigated. It is well accepted that preclinical testing is crucial for translation of novel developments into the clinic, but it is also criticized that with employing the rat sciatic nerve model also the surgery site is a protected one from an anatomical point of view (Kaplan et al. 2015). Any graft implanted along the thigh is, for example, not exposed to significant mechanical forces or bending (Kaplan et al. 2015). The latter condition is, however, important not only for long-graft repair as indicated in the most critical articles (Hoke 2006; Kaplan et al. 2015); it is also important when considering digital nerve repair. Digital nerve repair using nerve tubes is considered a valuable technique for which good outcome has been described, since it is easier to perform than autologous nerve grafting and does not need harvest of autologous donor tissue (Siemers and Houschyar 2017).

The second additional criticism is even more basic and will not be compensated by thoughtful modification of the study design in the rodent models. Overall, the regenerative potential and healing processes are accelerated and more homogenous in (laboratory rodent) research breeds in contrast to the diversity found in human patients (Kaplan et al. 2015), which probably also suffer from concomitant systemic diseases. The critique even goes further to the proposal of limiting the use of rat models in peripheral nerve regeneration studies to the clear evaluation of basic science questions (Kaplan et al. 2015). This proposal is based on the concern that with the deficiency in translatability, actually promising developments may be overseen, because they do not give good results in the rat models, while others will be overvalued for results achieved with parameters that do not appropriately mimic the conditions in human patients (Kaplan et al. 2015).

Although this critique is clearly worth consideration, still the second most used model for analyzing novel nerve graft constructs is the rabbit sciatic nerve. This model, however, represents with even more differences in limb and paw motion and function in comparison to human individuals than mouse or rat models (Angius et al. 2012), which makes functional evaluation and translatability more difficult and less robust. And also we still lack an optimally standardized animal model for objective evaluation of novel nerve graft developments in different laboratories (Angius et al. 2012). All this leads to the disillusioning perception that extrapolation of preclinical results toward the translational potential of an attempt for clinical use in human patients is considerably limited.

On the other hand, commercially available hollow nerve guidance conduits had so far regularly received clinical approval after they had been comprehensively studied in the rat sciatic nerve model for reconstructing a gap length of up to 15 mm. The latest example is Reaxon[®] Nerve Guide (Haastert-Talini et al. 2013; Gonzalez-Perez et al. 2015). And regulatory aspects rightly request evaluation of toxicity and biocompatibility that commonly need to be done in small animal models. Bioartificial tubular nerve implants mainly provide a single hollow lumen and are approved for clinical use in short nerve defect repair (<3 cm) only. And it is obvious that without additional modification, this kind of hollow nerve guidance channels will not become substitutes for autologous nerve grafts (Gu et al. 2014; Faroni et al. 2015). But the warranted modification will also increase the demands on regulatory work to be done before the application of novel developments in humans will be approved. For the tissue engineering field, this needs to be especially considered for cell-supplemented tissue-engineered products that are not exclusively composed of the patients' autologous cells, like so-called advanced therapy medicinal products (e.g., regulation on advanced therapies (Regulation (EC) 1394/2007) for the EU). Animal models for evaluating grafts supplemented with cells of human or other allogenic origins are today also restricted to the use of nude-rat strains since the implant will not be rejected by host-versus-graft immunogenicity (O'Rourke et al. 2018).

It is also true, however, that whenever clinical outcome after nerve reconstruction with the approved nerve guides has been analyzed in a prospective, randomized, and controlled way, the results have been variable from very good to poor and therefore certainly less convincing than in preclinical evaluation (Boeckstyns et al. 2013; Lohmeyer et al. 2014; Kaplan et al. 2015; Means et al. 2016; Neubrech et al. 2018). But again, until today no better preclinical models exist, and the author of this chapter is also aware of only one attempt in which a novel nerve graft development was comprehensively analyzed in the rat sciatic nerve model followed by a first-in-human study with repair of sural nerves after biopsy procedures (Bozkurt et al. 2017; van Neerven et al. 2017).

Therefore, it is well expectable that rat models will also be used in the future, but it should be more than obvious that the conclusions drawn from the same need to be well justified through elaborated study design and comprehensive data analyses.

4 Well-Thought-Out Rat Studies Provide Important Information After Comprehensive Functional Assessment

Taking into account the criticism pointed out above, it is, nevertheless, possible in rodent models to evaluate precisely the course from nerve injury to established functional recovery (Navarro 2016). This course of regeneration is divided in three general phases – elegantly described as the 3Rs of peripheral nerve regeneration – namely, axonal Regeneration, Reinnervation of peripheral targets, and functional Recovery (Navarro 2016). Each of those phases is dependent on the preceding one, and researchers should comprehensively apply the most objective methods for quantitative evaluation (Brushart 2011b, c; Navarro 2016). Additionally it needs to be considered that based on the huge prevalence of data available from rat studies (Angius et al. 2012; Geuna 2015; Gordon and Borschel 2017; Navarro 2016), new high-quality studies in this highly standardized animal model will allow better comparability among the work of different research groups. For this it will be of outmost importance that standard evaluation techniques are applied in a precise and objective way.

5 The Rat Sciatic Nerve Model

The rat sciatic nerve model enables evaluation of nerve graft properties for functional recovery after reconstruction of defects of 15–20 mm in length with a large variety of functional assessments (Brushart 2011c; Navarro 2016). Very often the complete sciatic nerve is transected and axonal regeneration is quantified using histological and functional readouts.

And it is also imperative not to use histomorphometrical evaluation of axonal regeneration across a certain distance as a stand-alone readout since it does not reliably predict the final functional outcome (Brushart 2011c). Also it is important that for histomorphometrical evaluation, unbiased and standardized techniques are applied in order to reveal the quality and quantity of regenerated axons. Researchers need to consider performing nerve morphometry on epoxy-embedded tissue sections (Geuna et al. 2004; Geuna et al. 2009; Raimondo et al. 2009) instead of simply performing vague evaluation of hematoxylin-eosin stained paraffin sections. Histology on paraffin-embedded tissue sections or even cryosections can, however, be a helpful additive tool for in-depth analysis of axonal populations or tissue interaction with the evaluated nerve graft development (Geuna et al. 2009; Haastert-Talini et al. 2013; Stenberg et al. 2017).

For generating a translation impact, nerve morphometry and in-depth histological evaluations need to be combined with at least one predictive assessment of functional recovery. Here again, histological methods could be helpful, e.g., proving target tissue reinnervation by retrograde labeling (Brushart 2011c), but many more groups would rely more on recording of evoked compound muscle action potentials (CMAPs) upon stimulation of the repaired nerve (Brushart 2011c; Navarro 2016).

Diverse readouts exist to determine in more detail the recovery of complex voluntary motor functions (e.g., video gait analysis (Costa et al. 2009)) as well as sensory recovery (e.g., von Frey mechanical pain threshold test) (Brushart 2011c; Navarro 2016). When exact muscle reinnervation is of relevance for a study, it may also be advantageous not to transect the complete sciatic nerve at mid-thigh level but to choose the common peroneal or the tibial branches supplying specific flexor and extensor muscles below the knee flexing or extending the ankle joint (Wood et al. 2011; Gordon and Borschel 2017).

Among the techniques for evaluating functional recovery, the calculation of the Sciatic Function Index (SFI, introduced in the early 1980s (de Medinaceli et al. 1982)) or the Static Sciatic Index (SSI, (Bozkurt et al. 2008)) from measured distances between the spread toes of the hind paws is certainly still the most commonly used one. In general, the index will indicate complete sciatic nerve dysfunction (no spreading of toes possible anymore) when calculation of its mathematical formula equates to -100 and full sciatic nerve function when the formula equates to zero (Navarro 2016). Variations of the test also consider function of isolated sciatic nerve branches, like the tibial nerve or common peroneal nerve (TFI, PFI, (Brushart 2011c)). Irrespective of its widespread use, the assessment of this kind of indexes loses reliability whenever a lesion, more severe than a crush lesion, is induced (Monte-Raso et al. 2008). While index values after crush injuries reliably return to pre-injury levels in reasonable time (Monte-Raso et al. 2008), obtainable values after repair of nerve transection, and especially after nerve graft repair, are often not valid to discriminate between implant properties, because substantial recovery is usually not reached (Navarro 2016). The failure of the test methods, especially after complete sciatic nerve transection injury, is mainly attributed to the innervation of alternative motor targets by misdirected regenerating axons resulting in contractures, joint stiffness, and abnormal paw posture in general (Brushart 2011c; Navarro 2016). Therefore, conclusions about the quality of novel nerve graft developments drawn on the basis of SFI or similar calculations and histomorphometrical analysis of regenerated axons at implant level should not be considered strong and predictive for clinical translatability.

The downsides of the complete sciatic nerve transection injury affecting useful evaluation of the SFI do also create other limitations of ethical concern. The injury model results in paralysis of the hind limb below the injury level and in sensation loss for the lateral part of the leg and paw. The model is often affiliated with automutilation behavior, meaning intensive licking, biting, and self-amputation of denervated digits and paws areas, and also with the abovementioned development of joint stiffness and hamstring contractures. All these represent events requiring at some level exclusion of the respective animal from a variety of functional tests but also early deliverance of the animal and its loss for the study. There have been attempts to specifically avoid automutilation behavior by administration of amitriptyline via the drinking water (Navarro et al. 1994) or via environmental enrichment, but the only reliable way of prevention is the choice of an appropriate rat strain (*please see below considerations on the rat strain*).

6 The Rat Median Nerve Model

Although the rat sciatic nerve injury and repair model enables functional assessment with diverse and also reliable, specific methods, the function of the nerve is different to that in human patients. Furthermore, peripheral nerve lesions to the upper limb show a higher prevalence than those to the lower limb in the clinic (Kouyoumdjian et al. 2017). Therefore, the rat median nerve injury and repair model is considered to be appropriate for extrapolation toward human upper limb or digital nerve injuries (Jager et al. 2014; Muratori et al. 2018; Ronchi et al. 2018; Dietzmeyer et al. 2019). With regard to ethical concerns, this model also represents with the advantage that lesion only partially impairs the upper limb function, because sensitivity and specific motor functions are preserved through innervation by the ulnar and the radial nerve (Bertelli et al. 1995; Sinis et al. 2006; Jager et al. 2014). With regard to translatability, it is also worth considering that hand function in humans requires fine finger movement which is similar to that in rats (Whishaw et al. 1992). Furthermore, it has recently been reinforced that the time course of stepwise functional recovery from electrodiagnosical detection of CMAPs, over skilled forelimb reaching until reflex-based grasping with a certain force, could be completely pictured in this model (Stossel et al. 2017). Considering the understandable concerns that long-gap repair and repair across graft challenging lesion sides may not be realized in the rat median nerve model, the author of this chapter wants to emphasize that a cross-chest repair paradigm could model regeneration distances of approximately 4 cm (Sinis et al. 2006). Besides the aforementioned electrodiagnostic testing and the evaluation of skilled forelimb reaching and reflex-based grafting, this model offers even more variety of functional assessment, including again walking track analysis (without the risk of automutilation), thermography, and nociception evaluation (Navarro 2016; Casal et al. 2018).

7 The Rat as Model Organism for Peripheral Nerve Repair Under Diabetic Conditions

Due to the possibility of genetic modification and induction of general health conditions modeling human systemic diseases, rat models will also be preserved in their prevalence for peripheral nerve regeneration studies. One example are studies performed in the Goto-Kakizaki rat with moderately increased and clinically relevant blood glucose levels and thus resembling diabetes type 2 conditions (Stenberg and Dahlin 2014; Stenberg et al. 2016). The model is, however, limited by a shorter life expectancy of these rats.

8 Other Species as Model Organism for Peripheral Nerve Repair: Addressing the Long Gap

With regard to long-gap repair models, certainly the sciatic nerve as the biggest nerve in the body already offers some variation of gap lengths when choosing alternative species for translational studies (Angius et al. 2012). As mentioned

above, the rabbit is often used as a model for longer gap repair, but certain limitations for studying functional recovery exist. Also the dog may represent a model (Wang et al. 2005; Ichihara et al. 2009; Okamoto et al. 2009; Ding et al. 2010; Yao et al. 2018), but ethical and economical concerns clearly limit its use in western countries for peripheral nerve regeneration studies. Considering that functional readouts should model functionality in humans as closely as possible, nonhuman primate models will obviously be the best to apply. Ethical concerns and economical concerns again represent the main restrictions for using nonhuman primates, and until today only a few studies exist in which recovery across a gap-length of up to 5 cm was studied after nerve graft repair (Archibald et al. 1995; Krarup et al. 2002; Hu et al. 2013; Pace et al. 2014; Fadia et al. 2020).

In recent years, the sheep has gained more interest for long-gap repair studies due to its economy and better ethical acceptance (Costa et al. 2018). The ovine model is also of interest due to the fact that the anatomy of ovine peripheral nerves models quite well that of human nerves in terms of length and diameter and functional impairment is not dramatically impacting the general condition of the animals (Diogo et al. 2017; Kornfeld et al. 2019). In the sheep a variety of nerves has been studied so far: fore and hind limb nerves but also the cranial and spinal nerves (Diogo et al. 2017). The model also enables evaluation of muscle function (Diogo et al. 2017), and also attempts to apply kinematic analysis of movement have been proposed (Costa et al. 2018). Finally, the life expectancy of sheep is significantly longer than that of laboratory rodents or rabbit, also enabling long-term evaluation. It will be up to the field to adopt this model more in the future and figure out if the ovine model will increase translation of novel tissue-engineered nerve grafts into the clinic.

9 Final Consideration of Appropriate Control Groups, Sex, Age, and Rat Strain of Choice

With regard to comparability and objectivity in evaluating the performance of novel nerve graft developments in rat models, it will always be imperative to include an experimental control group in which the reversed autologous nerve graft should be applied. If the gold standard control is not evaluated along with the new development, the author considers this a major ethical flaw, because animals are subjected to a study that does not challenge the new development to a maximum. In fact, from the author's point of view it can be justified to enroll a smaller animal number in such a group for reducing animal numbers involved in the study. It should still deliver sound standing data when, e.g., only four to six animals are subjected to autograft repair instead of more animals subjected to the other experimental repair groups. While the autograft control group is equally important for achieving relevant data and for not misusing animals in biomedical research, it is the author's personal opinion that it is a major ethical flaw to subject rats to sham surgery in peripheral nerve regeneration studies.

Regarding the consideration of gender in health research, the sex of animals subjected to preclinical studies in peripheral nerve regeneration may become more

considered in the future. For now researchers mainly decide for either male or female animals with no clear rationale besides size and handling issues, which are usually not clarified to the reader. Nevertheless, a few studies have been investigating sex differences in nerve regeneration in the rat. Therefore it has been described that supplemented testosterone and progesterone have an impact on the regeneration process and that levels of neuroactive steroids are different in male and female rats (Pesaresi et al. 2010; Melcangi et al. 2011). Also with interest in more general (neuro)pathological conditions, such as diabetes, it has been found that axonal outgrowth is longer in male than in female rats in the early phase of regeneration through tubular nerve grafts (Stenberg and Dahlin 2014). So far, a clear need to regularly compare regeneration outcome in male and female experimental animals cannot be concluded from the existing literature.

With regard to age, most studies in the field are conducted in young adult animals. For studying properties of novel nerve graft developments, it still seems appropriate to conduct peripheral nerve regeneration studies in young adult animals; irrespectively of demographic changes and the fact that retired people are at good health, considerably active, and therefore also at considerable risk to suffer from peripheral nerve lesion during leisure time (Eriksson et al. 2011). When studying nerve regeneration in aged animals, it has to be considered that the speed of axonal regeneration per se is most likely not reduced, but the process of Wallerian degeneration and thereby clearance of the distal nerve end is retarded (Kang and Lichtman 2013). Therefore, studies addressing the question of how to improve peripheral nerve regeneration in the elderly will currently not focus primarily on tissue engineering of nerve grafts but other approaches. For analyzing functional recovery, however, it may be an issue to make sure that performing complex tasks will crucially depend on the animals' learning willingness and ability, and also the completeness of the learning process (Stossel et al. 2017; Casal et al. 2018; Dietzmeyer et al. 2019) prior to starting the experiment should be reflected.

Finally, for studying aspects of functional recovery after peripheral nerve repair in the rat, it will be important to also consider the choice of the appropriate rat strain. Overall, with regard to outbred strains, the use of Sprague Dawley and Wistar rats appears to be predominant to the author of this chapter, while Lewis rats represent the predominantly used inbred strain (Angius et al. 2012). As, already mentioned above, events of automutilation could seriously impact the validity of a study, it should be considered that Lewis rats are the only rat strain known to be less prone to develop automutilation behavior (Carr et al. 1992; Shir et al. 2001). Consequently, when subjecting Lewis rats to sciatic nerve lesion and repair studies, it will also be more in line with the concept of the ethical 3Rs (Replacement, Reduction, Refinement (Tannenbaum and Bennett 2015)). Whenever the risk for exclusion of animals from a running study due to automutilation is minimized (Refinement), a lower number of animals (Reduction) can be subjected to the study (Stossel et al. 2018). But there is, however, also another aspect to be considered, and this refers to the motivation of the animals to participate in specific tests to be conducted in the awoken animal for estimation of functional recovery. With regard to the latter, Lewis rats may be less reliable in mechanical pain threshold evaluation in the sciatic

nerve injury and repair model (Webb et al. 2003). And also some more elaborate tests, like the staircase test evaluation of recovery of fine motor skills in the rat median nerve injury and repair model (Stossel et al. 2017), depend on the motivation of the animals. Again, Lewis rats have been described to probably be less motivated to participate and to also provide a potentially reduced learning ability (Nikkhah et al. 1998; Galtrey and Fawcett 2007).

10 Conclusions

The author of this chapter hopes that its content will increase awareness for thoughtful study design where the different possibilities for objective and effective evaluation are not yet considered enough and that this will bring research in the developing field of peripheral nerve engineering to a new level with increased probability for clinical translation of promising results.

References

- Angius D, Wang H, Spinner RJ, Gutierrez-Cotto Y, Yaszemski MJ, Windebank AJ (2012) A systematic review of animal models used to study nerve regeneration in tissue-engineered scaffolds. *Biomaterials* 33:8034–8039
- Archibald SJ, Shefner J, Krarup C, Madison RD (1995) Monkey median nerve repaired by nerve graft or collagen nerve guide tube. *J Neurosci* 15:4109–4123
- Bertelli JA, Taleb M, Saadi A, Mira JC, Pecot-Dechavassine M (1995) The rat brachial plexus and its terminal branches: an experimental model for the study of peripheral nerve regeneration. *Microsurgery* 16:77–85
- Boeckstyns ME, Sorensen AI, Vineta JF, Rosen B, Navarro X, Archibald SJ, Valss-Sole J, Moldovan M, Krarup C (2013) Collagen conduit versus microsurgical neuroorrhaphy: 2-year follow-up of a prospective, blinded clinical and electrophysiological multicenter randomized, controlled trial. *J Hand Surg Am* 38:2405–2411
- Bozkurt A, Tholl S, Wehner S, Tank J, Cortese M, O'Dey D, Deumens R, Lassner F, Schugner F, Groger A, Smeets R, Brook G, Pallua N (2008) Evaluation of functional nerve recovery with visual-SSI – a novel computerized approach for the assessment of the static sciatic index (SSI). *J Neurosci Methods* 170:117–122
- Bozkurt A, Deumens R, Beckmann C, Olde Damink L, Schugner F, Heschel I, Sellhaus B, Weis J, Jahnen-Dechent W, Brook GA, Pallua N (2009) In vitro cell alignment obtained with a Schwann cell enriched microstructured nerve guide with longitudinal guidance channels. *Biomaterials* 30:169–179
- Bozkurt A, Claeys KG, Schrading S, Rodler JV, Altinova H, Schulz JB, Weis J, Pallua N, van Neerven SGA (2017) Clinical and biometrical 12-month follow-up in patients after reconstruction of the sural nerve biopsy defect by the collagen-based nerve guide Neuromaix. *Eur J Med Res* 22:34
- Brushart TM (2011a) Preface. In: Brushart TM (ed) *Nerve repair*. Oxford University Press, New York, p vii
- Brushart TM (2011b) Determining experimental outcome. In: Brushart TM (ed) *Nerve repair*. Oxford University Press, New York, pp 135–158
- Brushart TM (2011c) Outcomes of experimental nerve repair and grafting. In: Brushart TM (ed) *Nerve repair*. Oxford University Press, New York, pp 159–195

- Carr MM, Best TJ, Mackinnon SE, Evans PJ (1992) Strain differences in autotomy in rats undergoing sciatic nerve transection or repair. *Ann Plast Surg* 28:538–544
- Casal D, Mota-Silva E, Iria I, Alves S, Farinho A, Pen C, Lourenco-Silva N, Mascarenhas-Lemos L, Silva-Ferreira J, Ferraz-Oliveira M, Vassilenko V, Videira PA, Goyri-O'Neill J, Pais D (2018) Reconstruction of a 10-mm-long median nerve gap in an ischemic environment using autologous conduits with different patterns of blood supply: a comparative study in the rat. *PLoS One* 13:e0195692
- Costa LM, Simoes MJ, Mauricio AC, Varejao AS (2009) Chapter 7: methods and protocols in peripheral nerve regeneration experimental research: part IV-kinematic gait analysis to quantify peripheral nerve regeneration in the rat. *Int Rev Neurobiol* 87:127–139
- Costa D, Diogo CC, Costa LMD, Pereira JE, Filipe V, Couto PA, Geuna S, Armada-Da-Silva PA, Mauricio AC, Varejao ASP (2018) Kinematic patterns for hindlimb obstacle avoidance during sheep locomotion. *Neurol Res* 40:963–971
- de Medinaceli L, Freed WJ, Wyatt RJ (1982) An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. *Exp Neurol* 77:634–643
- Dietzmeyer N, Forthmann M, Leonhard J, Helmecke O, Brandenberger C, Freier T, Haastert-Talini K (2019) Two-chambered chitosan nerve guides with increased bendability support recovery of skilled forelimb reaching similar to autologous nerve grafts in the rat 10 mm median nerve injury and repair model. *Front Cell Neurosci* 13:149
- Ding F, Wu J, Yang Y, Hu W, Zhu Q, Tang X, Liu J, Gu X (2010) Use of tissue-engineered nerve grafts consisting of a chitosan/poly(lactic-co-glycolic acid)-based scaffold included with bone marrow mesenchymal cells for bridging 50-mm dog sciatic nerve gaps. *Tissue Eng Part A* 16:3779–3790
- Diogo CC, Camassa JA, Pereira JE, Costa LMD, Filipe V, Couto PA, Geuna S, Mauricio AC, Varejao AS (2017) The use of sheep as a model for studying peripheral nerve regeneration following nerve injury: review of the literature. *Neurol Res* 39:926–939
- Eriksson M, Karlsson J, Carlsson KS, Dahlin LB, Rosberg HE (2011) Economic consequences of accidents to hands and forearms by log splitters and circular saws: cost of illness study. *J Plast Surg Hand Surg* 45:28–34
- Fadia NB, Bliley JM, DiBernardo GA, Crammond DJ, Schilling BK, Sivak WN, Spiess AM, Washington KM, Waldner M, Liao H-T, James IB, Minter DM, Tompkins-Rhoades C, Cottrill AR, Kim D-Y, Schweizer R, Bourne DA, Panagis GE, Schusterman II MA, Egro FM, Campwala IK, Simpson T, Weber DJ, Gause II T, Brooker JE, Josyula T, Guevara AA, Repko AJ, Mahoney CM, Marra KG (2020) Long-gap peripheral nerve repair through sustained release of a neurotrophic factor in nonhuman primates. *Sci Transl Med* 12:eaaav7753
- Faroni A, Mobasser SA, Kingham PJ, Reid AJ (2015) Peripheral nerve regeneration: experimental strategies and future perspectives. *Adv Drug Deliv Rev* 82–83:160–167
- Fields RD, Le Beau JM, Longo FM, Ellisman MH (1989) Nerve regeneration through artificial tubular implants. *Prog Neurobiol* 33:87–134
- Galtrey CM, Fawcett JW (2007) Characterization of tests of functional recovery after median and ulnar nerve injury and repair in the rat forelimb. *J Peripher Nerv Syst* 12:11–27
- Geuna S (2015) The sciatic nerve injury model in pre-clinical research. *J Neurosci Methods* 243:39–46
- Geuna S, Gigo-Benato D, Rodrigues Ade C (2004) On sampling and sampling errors in histomorphometry of peripheral nerve fibers. *Microsurgery* 24:72–76
- Geuna S, Raimondo S, Ronchi G, Di Scipio F, Tos P, Czaja K, Fornaro M (2009) Chapter 3: histology of the peripheral nerve and changes occurring during nerve regeneration. *Int Rev Neurobiol* 87:27–46
- Geuna S, Gnani S, Perroteau I, Tos P, Battiston B (2013) Tissue engineering and peripheral nerve reconstruction: an overview. *Int Rev Neurobiol* 108:35–57
- Geuna S, Raimondo S, Fregnan F, Haastert-Talini K, Grothe C (2016) In vitro models for peripheral nerve regeneration. *Eur J Neurosci* 43:287–296

- Gonzalez-Perez F, Cobiañchi S, Geuna S, Barwig C, Freier T, Udina E, Navarro X (2015) Tubulization with chitosan guides for the repair of long gap peripheral nerve injury in the rat. *Microsurgery* 35(4):300–308
- Gordon T, Borschel GH (2017) The use of the rat as a model for studying peripheral nerve regeneration and sprouting after complete and partial nerve injuries. *Exp Neurol* 287:331–347
- Grasman JM, Ferreira JA, Kaplan DL (2018) Tissue models for neurogenesis and repair in 3D. *Adv Funct Mater* 2018:1803822
- Grinsell D, Keating CP (2014) Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. *Biomed Res Int* 2014:698256
- Gu X, Ding F, Williams DF (2014) Neural tissue engineering options for peripheral nerve regeneration. *Biomaterials* 35:6143–6156
- Haastert-Talini K, Geuna S, Dahlin LB, Meyer C, Stenberg L, Freier T, Heimann C, Barwig C, Pinto LF, Raimondo S, Gambarotta G, Samy SR, Sousa N, Salgado AJ, Ratzka A, Wrobel S, Grothe C (2013) Chitosan tubes of varying degrees of acetylation for bridging peripheral nerve defects. *Biomaterials* 34:9886–9904
- Hoke A (2006) Mechanisms of disease: what factors limit the success of peripheral nerve regeneration in humans? *Nat Clin Pract Neurol* 2:448–454
- Hu N, Wu H, Xue C, Gong Y, Wu J, Xiao Z, Yang Y, Ding F, Gu X (2013) Long-term outcome of the repair of 50 mm long median nerve defects in rhesus monkeys with marrow mesenchymal stem cells-containing, chitosan-based tissue engineered nerve grafts. *Biomaterials* 34:100–111
- Ichihara S, Inada Y, Nakada A, Endo K, Azuma T, Nakai R, Tsutsumi S, Kurosawa H, Nakamura T (2009) Development of new nerve guide tube for repair of long nerve defects. *Tissue Eng Part C Methods* 15:387–402
- Jager SB, Ronchi G, Vaegter CB, Geuna S (2014) The mouse median nerve experimental model in regenerative research. *Biomed Res Int* 2014:701682
- Kang H, Lichtman JW (2013) Motor axon regeneration and muscle reinnervation in young adult and aged animals. *J Neurosci* 33:19480–19491
- Kaplan HM, Mishra P, Kohn J (2015) The overwhelming use of rat models in nerve regeneration research may compromise designs of nerve guidance conduits for humans. *J Mater Sci Mater Med* 26:226
- Kornfeld T, Vogt PM, Radtke C (2019) Nerve grafting for peripheral nerve injuries with extended defect sizes. *Wien Med Wochenschr* 169:240–251
- Kouyoumdjian JA, Graca CR, Ferreira VFM (2017) Peripheral nerve injuries: a retrospective survey of 1124 cases. *Neurol India* 65:551–555
- Krarup C, Archibald SJ, Madison RD (2002) Factors that influence peripheral nerve regeneration: an electrophysiological study of the monkey median nerve. *Ann Neurol* 51:69–81
- Lohmeyer JA, Kern Y, Schmauss D, Paprottka F, Stang F, Siemers F, Mailaender P, Machens HG (2014) Prospective clinical study on digital nerve repair with collagen nerve conduits and review of literature. *J Reconstr Microsurg* 30:227–234
- Means KR Jr, Rinker BD, Higgins JP, Payne SH Jr, Merrell GA, Wilgis EF (2016) A multicenter, prospective, randomized, pilot study of outcomes for digital nerve repair in the hand using hollow conduit compared with processed allograft nerve. *Hand (N Y)* 11:144–151
- Melcangi RC, Giatti S, Pesaresi M, Calabrese D, Mitro N, Caruso D, Garcia-Segura LM (2011) Role of neuroactive steroids in the peripheral nervous system. *Front Endocrinol (Lausanne)* 2:104
- Mobini S, Song YH, McCrary MW, Schmidt CE (2019) Advances in ex vivo models and lab-on-a-chip devices for neural tissue engineering. *Biomaterials* 198:146–166
- Monte-Raso VV, Cláudio Henrique Barbieri CH, Mazzer N, Calura A (2008) Is the sciatic function index always reliable and reproducible? *J Neurosci Methods* 170:255–261
- Muratori L, Gnani S, Fregnan F, Mancardi A, Raimondo S, Perroteau I, Geuna S (2018) Evaluation of vascular endothelial growth factor (VEGF) and its family member expression after peripheral nerve regeneration and denervation. *Anat Rec (Hoboken)* 301:1646–1656

- Navarro X (2016) Functional evaluation of peripheral nerve regeneration and target reinnervation in animal models: a critical overview. *Eur J Neurosci* 43:271–286
- Navarro X, Buti M, Verdu E (1994) Autotomy prevention by amitriptyline after peripheral nerve section in different strains of mice. *Restor Neurol Neurosci* 6:151–157
- Neubrech F, Sauerbier M, Moll W, Seegmuller J, Heider S, Harhaus L, Bickert B, Kneser U, Kremer T (2018) Enhancing the outcome of traumatic sensory nerve lesions of the hand by additional use of a chitosan nerve tube in primary nerve repair: a randomized controlled bicentric trial. *Plast Reconstr Surg* 142:415–424
- Nikkhah G, Rosenthal C, Hedrich HJ, Samii M (1998) Differences in acquisition and full performance in skilled forelimb use as measured by the ‘staircase test’ in five rat strains. *Behav Brain Res* 92:85–95
- O’Rourke C, Day AGE, Murray-Dunning C, Thanabalasundaram L, Cowan J, Stevanato L, Grace N, Cameron G, Drake RAL, Sinden J, Phillips JB (2018) An allogeneic ‘off the shelf’ therapeutic strategy for peripheral nerve tissue engineering using clinical grade human neural stem cells. *Sci Rep* 8:2951
- Okamoto H, Hata KI, Kagami H, Okada K, Ito Y, Narita Y, Hirata H, Sekiya I, Otsuka T, Ueda M (2009) Recovery process of sciatic nerve defect with novel bioabsorbable collagen tubes packed with collagen filaments in dogs. *J Biomed Mater Res A* 92:859–868
- Pace LA, Plate JF, Mannava S, Barnwell JC, Koman LA, Li Z, Smith TL, Van Dyke M (2014) A human hair keratin hydrogel scaffold enhances median nerve regeneration in nonhuman primates: an electrophysiological and histological study. *Tissue Eng Part A* 20:507–517
- Pesaresi M, Maschi O, Giatti S, Garcia-Segura LM, Caruso D, Melcangi RC (2010) Sex differences in neuroactive steroid levels in the nervous system of diabetic and non-diabetic rats. *Horm Behav* 57:46–55
- Raimondo S, Fornaro M, Di Scipio F, Ronchi G, Giacobini-Robecchi MG, Geuna S (2009) Chapter 5: methods and protocols in peripheral nerve regeneration experimental research: part II- morphological techniques. *Int Rev Neurobiol* 87:81–103
- Ronchi G, Fornasari BE, Crosio A, Budau CA, Tos P, Perroteau I, Battiston B, Geuna S, Raimondo S, Gambarotta G (2018) Chitosan tubes enriched with fresh skeletal muscle fibers for primary nerve repair. *Biomed Res Int* 2018:9175248
- Schiefer JL, Schulz L, Rath R, Stahl S, Schaller HE, Manoli T (2015) Comparison of short- with long-term regeneration results after digital nerve reconstruction with muscle-in-vein conduits. *Neural Regen Res* 10:1674–1677
- Shir Y, Zeltser R, Vatine JJ, Carmi G, Belfer I, Zangen A, Overstreet D, Raber P, Seltzer Z (2001) Correlation of intact sensibility and neuropathic pain-related behaviors in eight inbred and outbred rat strains and selection lines. *Pain* 90:75–82
- Siddique R, Vyas A, Thakor N, Brushart TM (2014) A two-compartment organotypic model of mammalian peripheral nerve repair. *J Neurosci Methods* 232:84–92
- Siemers F, Houschyar KS (2017) Alternative strategies for nerve reconstruction. In: Haastert-Talini K, Assmus H, Antoniadis G (eds) *Modern concepts of nerve repair*. Springer International Publishing AG, Cham, pp 79–96
- Siemionow M, Bozkurt M, Zor F (2010) Regeneration and repair of peripheral nerves with different biomaterials: review. *Microsurgery* 30:574–588
- Sinis N, Schaller HE, Becker ST, Lanaras T, Schulte-Eversum C, Muller HW, Vonthein R, Rosner H, Haerle M (2006) Cross-chest median nerve transfer: a new model for the evaluation of nerve regeneration across a 40 mm gap in the rat. *J Neurosci Methods* 156:166–172
- Siqueira MG, Kline DG (2018) Sir Sydney Sunderland and the Sunderland society. *J Neurosurg* 129:1325–1330
- Siqueira MG, Martins RS (2017) Conventional strategies for nerve repair. In: Haastert-Talini K, Assmus H, Antoniadis G (eds) *Modern concepts of nerve repair*. Springer International Publishing AG, Cham, pp 41–51
- Stenberg L, Dahlin LB (2014) Gender differences in nerve regeneration after sciatic nerve injury and repair in healthy and in type 2 diabetic Goto-Kakizaki rats. *BMC Neurosci* 15:107

- Stenberg L, Kodama A, Lindwall-Blom C, Dahlin LB (2016) Nerve regeneration in chitosan conduits and in autologous nerve grafts in healthy and in type 2 diabetic Goto-Kakizaki rats. *Eur J Neurosci* 43:463–473
- Stenberg L, Stossel M, Ronchi G, Geuna S, Yin Y, Mommert S, Martensson L, Metzen J, Grothe C, Dahlin LB, Haastert-Talini K (2017) Regeneration of long-distance peripheral nerve defects after delayed reconstruction in healthy and diabetic rats is supported by immunomodulatory chitosan nerve guides. *BMC Neurosci* 18:53
- Stossel M, Rehra L, Haastert-Talini K (2017) Reflex-based grasping, skilled forelimb reaching, and electrodiagnostic evaluation for comprehensive analysis of functional recovery—the 7-mm rat median nerve gap repair model revisited. *Brain Behav* 7:e00813
- Stossel M, Wildhagen VM, Helmecke O, Metzen J, Pfund CB, Freier T, Haastert-Talini K (2018) Comparative evaluation of chitosan nerve guides with regular or increased bendability for acute and delayed peripheral nerve repair: a comprehensive comparison with autologous nerve grafts and muscle-in-vein grafts. *Anat Rec (Hoboken)* 301:1697–1713
- Tannenbaum J, Bennett BT (2015) Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose. *J Am Assoc Lab Anim Sci* 54:120–132
- Tos P, Ronchi G, Papalia I, Sallen V, Legagneux J, Geuna S, Giacobini-Robecchi MG (2009) Chapter 4: methods and protocols in peripheral nerve regeneration experimental research: part I—experimental models. *Int Rev Neurobiol* 87:47–79
- van Neerven SGA, Haastert-Talini K, Boecker A, Schriever T, Dabhi C, Claeys K, Deumens R, Brook GA, Weis J, Pallua N, Bozkurt A (2017) Two-component collagen nerve guides support axonal regeneration in the rat peripheral nerve injury model. *J Tissue Eng Regen Med* 11:3349–3361
- Vyas A, Li Z, Aspalter M, Feiner J, Hoke A, Zhou C, O'Daly A, Abdullah M, Rohde C, Brushart TM (2010) An in vitro model of adult mammalian nerve repair. *Exp Neurol* 223:112–118
- Wang X, Hu W, Cao Y, Yao J, Wu J, Gu X (2005) Dog sciatic nerve regeneration across a 30-mm defect bridged by a chitosan/PGA artificial nerve graft. *Brain* 128:1897–1910
- Webb AA, Gowribai K, Muir GD (2003) Fischer (F-344) rats have different morphology, sensorimotor and locomotor abilities compared to Lewis, Long-Evans, Sprague-Dawley and Wistar rats. *Behav Brain Res* 144:143–156
- Whishaw IQ, Pellis SM, Gorny BP (1992) Skilled reaching in rats and humans: evidence for parallel development or homology. *Behav Brain Res* 47:59–70
- Wood MD, Kemp SW, Weber C, Borschel GH, Gordon T (2011) Outcome measures of peripheral nerve regeneration. *Ann Anat* 193:321–333
- Yao Y, Cui Y, Zhao Y, Xiao Z, Li X, Han S, Chen B, Fang Y, Wang P, Pan J, Dai J (2018) Effect of longitudinally oriented collagen conduit combined with nerve growth factor on nerve regeneration after dog sciatic nerve injury. *J Biomed Mater Res B Appl Biomater* 106:2131–2139



Basic Nerve Histology and Histological Analyses Following Peripheral Nerve Repair and Regeneration

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Abstract

In peripheral nerve studies, histological analyses represent one of the most widely used and informative quality controls to demonstrate nerve tissue regeneration. These analyses require several technical procedures, and a basic knowledge of nerve histology and regeneration is needed for the correct interpretation of the histological information obtained from the experimental studies. For this reason, the aim of this chapter is to review the basic nerve histology and regeneration, as well as the technical procedures and stainings available for the assessment of nerve tissue regeneration. In this sense, some concepts and technical details of the nerve tissue fixation and processing for light and electron microscopy are comprehensively discussed. In relation to histological staining, they were reviewed according to their application in the assessment of nerve tissue regeneration, stromal remodeling, and host response following nerve repair and regeneration. Finally, the importance and main advantages offered by the quantitative analyses of nerve regeneration, mainly focused on the semithin and ultrastructural analyses, were reported.

1 Introduction

Histology is considered one of the most useful and accurate quality controls in tissue engineering, including the assessment of peripheral nerve regeneration along bioengineered substitutes (Carriel et al. [2014b](#), [2017a](#)). Histology comprises a series of technical procedures and offers a wide range of possibilities to evaluate the cellular and molecular processes that occur during peripheral nerve regeneration. With the aim to provide a complete overview of histological techniques, this chapter is organized in four main sections. First, a basic introduction to peripheral nerve histology, including the degeneration and regeneration process, is provided. Second, the most relevant technical considerations for the histological preparation of samples are discussed. Third, a comprehensive review and analysis of the histological stainings available for the assessment of the peripheral nerve tissue regeneration and related process are presented, culminating with some recommendations and conclusions.

2 Basic Histology, Degeneration, and Nerve Tissue Regeneration

2.1 Basic Histology

Peripheral nerves (PNs) are delicate organs of the peripheral nervous system that functionally connect the central nervous system (CNS) with distal target organs creating an extremely complex, but well-organized circuit through the whole

body (Mills 2007). From the functional point of view, these organs are specialized in the conduction of electrical nerve impulses which allow the CNS to coordinate the motor and sensory functions of the body (Carriel et al. 2014c). The peripheral motor function is subdivided into somatic or voluntary nervous system and autonomic (vegetative) or involuntary nervous system. The latter is divided into sympathetic and parasympathetic nervous systems, respectively (Junqueira and Carneiro 2005; Welsch et al. 2009; Kierszenbaum and Tres 2016; Gartner 2017).

To carry out these functions, PNs possess a complex histological structure and molecular composition (Carriel et al. 2014a). Histologically, PNs are organized in two well-defined tissues: the nerve tissue or parenchyma and connective tissue or stroma (Mills 2007; Geuna et al. 2009). A schematic diagram of the stromal and parenchyma organization can be found in Fig. 1.

2.1.1 The Parenchyma

The *parenchyma* represents the functional tissue of these organs, and it is organized in highly specialized conductive units, the PN fibers (PNFs). These complex structures are responsible to relay information to and from CNS through afferent (sensitive) and efferent (motor) nerve impulses (Mills 2007; Geuna et al. 2009; Mescher 2018). Structurally, PNFs are composed of the neural axon, its surrounding Schwann cell (SC), and an external basal membrane (BM) (Mills 2007).

Axons are internally composed of a small amount of actin microfilaments, abundant and parallel aligned neurofilaments (NFLs) and neurotubules or microtubules (MTs) (Mills 2007). NFLs are type IV neuronal-specific intermediate filaments, and there are three types (light, medium, and heavy molecular weight mass) (Kevenaar and Hoogenraad 2015). The MTs are polymers composed of globular tubulin (a heterodimer of α and β tubulin) subunits which form tubular nanostructures, the neurotubules. Neurotubules are indispensable for the bidirectional axonal transport. Although MTs are not neuronal specific, they are often stained by immunohistochemical (IHC) methods for the study of neurons (body, dendrites, and axons) (Arroyo and Scherer 2000; Mills 2007).

According to the mechanism of interaction of the SCs with the axons, PNFs can be myelinated or unmyelinated (m-PNFs and u-PNFs, respectively) with important physiological implications (Arroyo and Scherer 2000). Indeed, myelin sheaths reduce the ion capacitance along the axoplasmic membrane resulting in a saltatory and fast conduction of the nerve impulses (Arroyo and Scherer 2000; Mills 2007; Welsch and Sobotta 2008; Geuna et al. 2009).

The m-PNFs result from the interaction of a unique SC with a specific axonal segment. During myelination, SCs progressively wrap the axonal segment, the cell membrane progressively compresses and finally a multilayered myelin sheath is formed as well as the internal and external mesaxon (Fawcett and Jensch 1997; Geuna et al. 2009; Kierszenbaum 2019). The myelin itself is largely composed of a heterogeneous group of lipids, such as cholesterol, phospholipids, galactolipids, and plasmalogen (70–75%), and some proteins (25%) (Fernandez et al. 2016). Regarding the myelin proteins, there is a set of unique molecules such as myelin

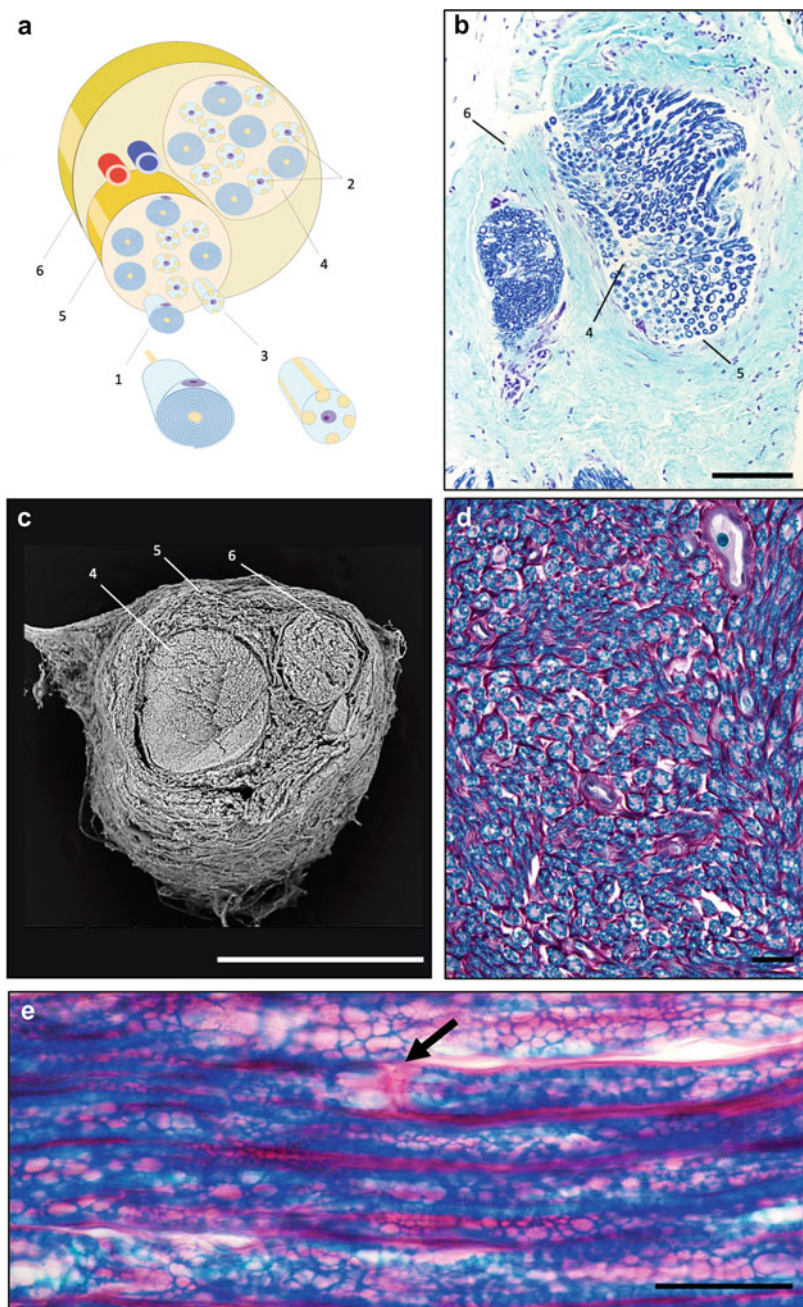


Fig. 1 Histological features of peripheral nerves. (a) Schematic representation of the nerve stroma and parenchyma organization in a bifasciculated nerve. (b) Transverse histological section of a rat sciatic nerve stained with Luxol fast blue which stains myelin blue, background light green,

protein 0 (P0), peripheral myelin protein 22 (PMP22), myelin basic protein (MBP), and the myelin-associated glycoprotein (MAG) (Arroyo and Scherer 2000; Mills 2007).

Transmission electron microscopy (TEM) allows an uncompacted region, the Schmidt-Lanterman incisures, to be identified along the myelin sheaths (Spiegel and Peles 2002; Pavelka and Roth 2010). These oblique interruptions form a fast route for the passage of substances through the myelin sheet layers (Arroyo and Scherer 2000; Meier et al. 2004). Independent myelin sheaths are separated by the nodes of Ranvier (NR), an ion channel-rich region, where axons are covered by small interdigitations of the adjacent SCs (the paranodal loops and nodal microvilli). The axonal segment covered by the myelin sheaths are also known as the intermodal segments, which can be identified with some myelin stainings in light microscopy (Fig. 1) (Mills 2007; Geuna et al. 2009; Pavelka and Roth 2010).

The u-PNFs are composed of several small neuronal axons surrendered by a unique SC invested by a BM. During development, a group of tiny axons are enveloped by a sequential series of SCs. These axons are progressively reorganized in the SC cytoplasm and finally they are separated from each other by SC cytoplasmic tongues which form a mesaxon in each (Mills 2007). Axonal surface is separated from the SC membrane by the periaxonal space of Klebs which allows the diffusion of ions when an action potential takes place (Griffin and Thompson 2008). The SCs associated with unmyelinated axons are often referred to as Remak cells as Robert Remak was the first to describe them in 1838. (Mills 2007; Griffin and Thompson 2008; Geuna et al. 2009). Moreover, u-PNFs are also known as Remak bundles.

Myelinating SC and Remak cells are similar, but differ in the expression of some proteins. In general, both cells express vimentin, S-100 protein, surface molecules (CD56, CD57, and CD146), and BM-associated molecules. Nevertheless, Remak cells tend to express glial fibrillar acid protein (GFAP) and lack MAG and other myelin-related proteins (Mills 2007; Philips et al. 2018b).

The PNF BM, like others, is composed of a set of fibrillar and nonfibrillar extracellular matrix molecules (ECM), which form a highly organized tubular structure, the endoneurial tubes, around PNFs (Topp and Boyd 2006; Philips et al. 2018b). Molecularly, the BM is composed of laminin isoforms, entactin/nidogen, fibronectin, heparin sulfate, N-syndecan, and collagens (types I, III, IV, and V) (Mills 2007; Philips et al. 2018b). This structure is crucial for the normal function of PNFs, and during regeneration, these molecules, such as laminin, play a fundamental role in guiding the regeneration process.



Fig. 1 (continued) and mast cells granules purple (metachromatic reaction). Scale bar = 100µm. (c) Scanning electron microscopy of a transverse section of rat sciatic nerve. Scale bar = 1000µm. (d) and (e) transverse and longitudinal sections of rat sciatic nerve stained with MCOLL staining (from Myelin and Collagen staining). Note the intense histochemical reaction for myelin in blue, and the endoneurial collagen content in red. Scale bar = 20µm. 1 = m-PNF, 2 = SC nuclei, 3 = u-PNF, 4 = endoneurium, 5 = perineurium, 6 = epineurium, Ranvier node (arrow)

Most PNs contain a mixture of myelinated and unmyelinated PNFs which are evident in transverse sections of nerves, especially in semithin and ultrathin sections. Mixed nerve histology is characterized by the presence of a heterogeneous population of PNFs which vary widely in PNF diameter, axon diameter, and myelin sheath thickness (Mills 2012).

2.1.2 The Stroma

While the parenchyma has been the focus of research for many years, the importance of peripheral nerve stroma has been less explored. The stroma is composed of specialized connective tissues which not only provide structure, mechanical strength, and protection to these organs, but are also the source of blood supply, and immunological and protective barriers (Topp and Boyd 2006; Mills 2007; Geuna et al. 2009; Peltonen et al. 2013).

In transverse histological sections of PNs, it is easily observable that PNFs are grouped and surrounded by a small amount of a loose connective tissue, the endoneurium. The endoneurial compartment is well delimited by a thin, dense, and highly specialized layer, the perineurium, which delimits the PN fascicles. The number and dimensions of these fascicles vary according to the PN's anatomical location. Individual or several nerve fascicles are surrounded by an external vascularized connective tissue layer, the epineurium (Topp and Boyd 2006; Mills 2007; Welsch and Sobotta 2008; Geuna et al. 2009).

The *epineurium* is a relatively dense vascularized connective tissue layer which plays an essential role in the biomechanical behavior of these organs (Topp and Boyd 2006). This layer is mainly composed of collagen fibers (types I, II, and III), a variable amount of elastic fibers and adipose tissue (Topp and Boyd 2006; Geuna et al. 2009). The 3D organization of the fibrillar ECM molecules allows some degree of torsion but efficiently limits overdistension and tearing of PNs during exercise (Topp and Boyd 2006; Mills 2007; Geuna et al. 2009). In this layer, there is a heterogeneous population of cells, the fibroblasts being the most abundant. Other cell types that can be observed are adipocytes, mast cells, and dendritic cells (Giorgadze et al. 2010).

The *perineurium* is a highly specialized stromal layer that extends from the CNS and ends at the level of encapsulated nerve endings (Welsch and Sobotta 2008). Although the perineurium is the thinnest stromal layer of these organs, it is determinant for the biomechanical behavior and resistance of PNs (Topp and Boyd 2006; Philips et al. 2018b). It is composed of alternating perineurial cells and ECM layers, which through its complex 3D organization and cell-cell and cell-ECM interactions constitute the peripheral nerve barrier or perineurial barrier (Peltonen et al. 2013). Perineurial cells are flattened and establish well-formed tight junctions that contain occludins, zonula occludent-1 (ZO-1), and claudin 1 and 3 (CLDN1 and CLDN3), which confer the barrier functions (Peltonen et al. 2013). Moreover, each cell layer is surrounded by a prominent double BM which, in larger PNs, can be up to 500µm thick, providing additional molecular components to the perineurial barrier (Topp and Boyd 2006; Peltonen et al. 2013). Between the perineurial cell layers and BM there are collagens (type I, II, and III) and elastic fibers organized in circumferential, oblique, and longitudinal sheets (Welsch and Sobotta 2008; Godoy-Guzman et al. 2018). Interestingly, epineurium and

perineurium are connected by a loose ECM which facilitates the sliding of one fascicle independently of adjacent ones (Topp and Boyd 2006; Mills 2007). Perineural cells express vimentin, tight junction proteins, cytokeratin 18, and the epithelial membrane antigen (EMA) (Rodriguez et al. 2012; Marettová 2016).

The *endoneurium* is the loose connective tissue found in the inner compartment of PN fascicles. This tissue provides an adequate microenvironment for the parenchyma function. The endoneurial ECM is composed of collagen fibers, elastic fibers, and some recently described proteoglycans (decorin, versican, and fibromodulin) (Mills 2007; Godoy-Guzman et al. 2018). This ECM is organized in endoneurial tubes which are composed of two distinct sheaths (Mills 2007). The outer membrane is composed of longitudinally oriented collagen fibers and the inner endoneurial sheath is composed of circumferentially or oblique oriented collagen fibers just outside the PNF BM. In the endoneurial compartment of some large species, like primates and humans, it is possible to find the Renault bodies. Although their function is not clear, it is hypothesized that they could provide mechanical protection to the parenchyma from compressive forces (Mills 2007; Pina-Oviedo et al. 2009).

Nerve vascularization enters through the epineurium where segmental arteries emit branches along nerve length to form the vasa nervorum (Welsch et al. 2009). These branches divide into large and mainly longitudinal blood vessels that form a network of epineurial arterioles. These arterioles send branches, characterized by a poorly developed muscular layer, that cross the perineurium. Once in the endoneurium, the blood vessels immediately turn into large-diameter and longitudinally oriented capillaries (Topp and Boyd 2006; Ubogu 2013). The ECM, endothelial cells, and pericytes establish the blood-nerve barrier (Topp and Boyd 2006; Geuna et al. 2009; Ubogu 2013).

2.2 Degeneration and Regeneration of Peripheral Nerves

The structure and function of PNs can be affected by diverse pathologies which fall into two main categories: peripheral neuropathies and structural injuries. Neuropathies can be associated with axonal degeneration or different kind of demyelination processes. The structural PN injuries are frequently caused by a wide range of traumatic injuries, but also due to the surgical removal of diverse neoplasm (Oh 2002; Lundborg 2004; Thorsen et al. 2012). Following structural damage, the PNs have an acceptable capability to regenerate their stroma and parenchyma. However, the degree of functional recovery, thus the neuronal and axonal fates, will be conditioned by the severity of the damage (short or critical nerve gaps), the histological structures preserved, the surgical treatment used, and the regenerative microenvironment (de Ruiter et al. 2014). More details about surgical repair strategies can be found in chapter “Surgical Techniques in Nerve Repair.”

After traumatic PN injuries, complex pathophysiologic changes occur at the neuron body and injury site (proximal nerve stump, interstump gap, and distal segment). These changes are briefly described below and schematically illustrated in Fig. 2.

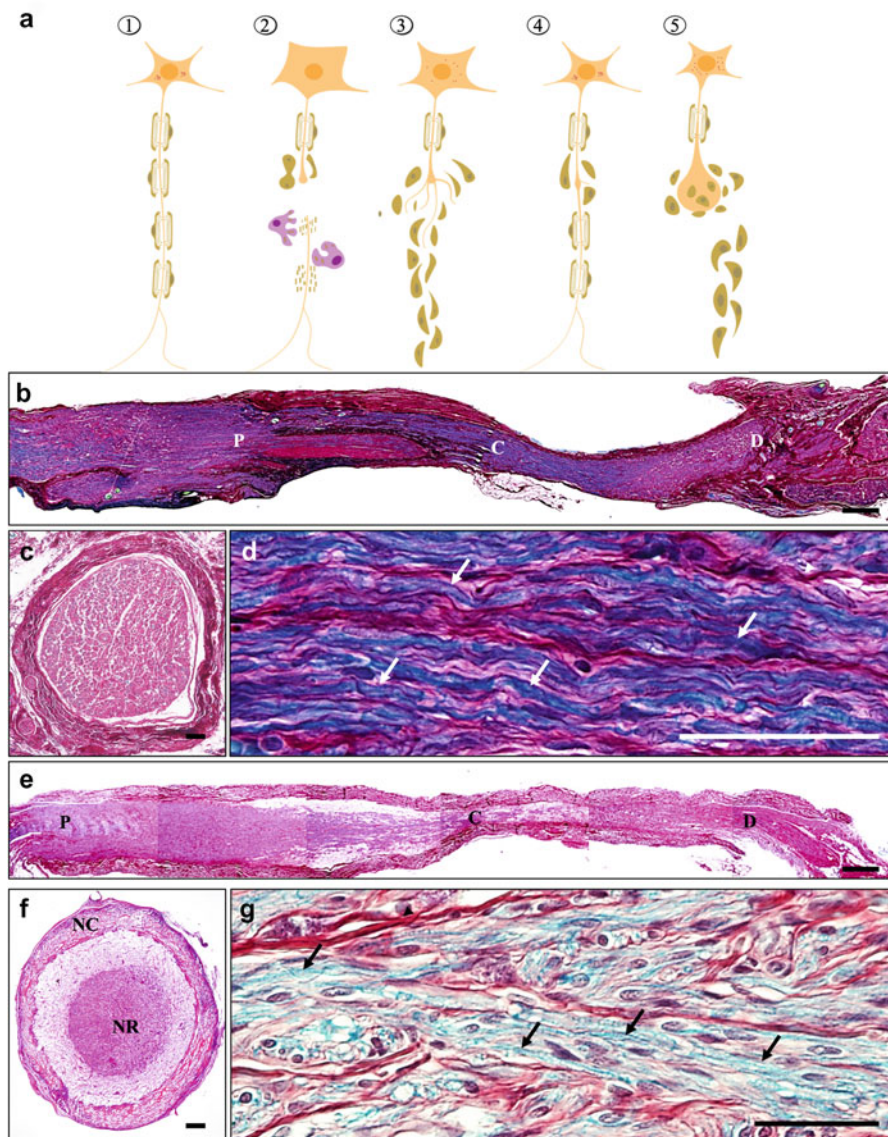


Fig. 2 Peripheral nerve degeneration and regeneration. (a) Graphical representation of morphological changes after PN injury. 1 = Normal structural configuration of a myelinated neuron associated with different SCs; 2 = After nerve transection, the neuronal body increases in volume and the distal segment undergoes WD; 3 = Terminal sprouts spontaneously arise from the proximal end as the SCs proliferate and guide them to the target organ; 4 = When reconnection of the distal organs is achieved maturation and consolidation occur; 5 = When the growth cones do not reach the distal nerve segment permanent transection of the nerve can lead to neuroma formation. B-G images show examples of nerve regeneration with MCOLL staining. B-D images show the histological aspects of peripheral nerve regeneration along an acellular nerve allograft longitudinally (b and d),

2.2.1 Neuron Body Response to Peripheral Nerve Injury

Following PN transection, the axon distal to the lesion site disconnects from the cell body. This fact induces a morphofunctional transformation in the neuronal bodies from a functional state (synthesis, transport, and storage of neurotransmitters; perception and conduction of nerve impulses) to a regenerative phenotype (to promote axonal regeneration, migration, and distal targets reinnervation) (Lundborg 2004; Geuna et al. 2009). The typical response is characterized by an increase of cell body volume accompanied by nuclear eccentricity, retraction of dendrites, and disorganization (chromatolysis) of Nissl bodies (Geuna et al. 2009). The metabolic changes that occur in the cell body lead to a decrease in the synthesis of neurotransmission-related products and an increased synthesis of growth-associated proteins and components of the cell structure. In this complex process, hundreds of genes are upregulated and downregulated (Navarro 2009) with ion and excitability changes. In this sense, different kinds of sodium channels are inserted into the membrane that contribute to the electrical hyperexcitability and ectopic electric discharges (in the injury site and dorsal root ganglia neurons) (Sun et al. 2005; Geuna et al. 2009; Navarro 2009).

2.2.2 Changes from the Proximal to the Distal Nerve Stumps

In the *proximal segment*, the axons degenerate from the lesion site to some distance back, in a process known as retrograde degeneration. During this process, the axons retract over one or several internodal segments leaving behind the endoneurial compartment as empty cylinders or endoneurial tubes (Geuna et al. 2009). After some hours, from the tip of the sectioned axon and from the NR of a noninjured axon, terminal and collateral sprouts spontaneously arise that quickly degenerate (Gordon 2009). The time required for the definitive sprouts to appear has been called initial delay and it is accompanied by a structural cytoskeletal stabilization. The axonal sprouting process is highly complex with an intrinsic variable behavior, where direct, lateral, and arborizing projections can be found in the same regeneration model. After the sprouting, axons advance in growth cones guided by the SC and ECM molecules (the BM and/or ECM endoneurial tubes) until they reinnervate the target. Following the innervation, the axon enlarges radially to its mature diameter with a progressive cytoskeletal maturation where the growth associating protein 43 (GAP-43) plays an essential role (Lundborg 2004; Geuna et al. 2009; Navarro 2009; Carriel et al. 2014c).



Fig. 2 (continued) and transversally (c) oriented. Images E-G show the nerve tissue regeneration inside a collagen-based nerve conduit, longitudinally (e, g) and transversally oriented (f). Note the signs of myelination (blue histochemical reaction indicated with arrows) and the collagen fibers (red histochemical reaction) in Figs. B, D, and G. NC = nerve conduit; NR = nerve regeneration; P = proximal nerve stump; C = central nerve stump, and D = distal nerve stump. Scale bars: 500µm (B and E), 100µm (C and F), 50µm (D and G). Images E) and G) were reproduced from Carriel et al. 2011a, 2017a with permission from Springer

Once the axon-SC interaction is interrupted, the myelin sheath is degraded by macrophages and the SCs start to dedifferentiate, proliferate, and actively guide axonal regeneration. The proliferation starts from both nerve stumps and, as a result, SCs form longitudinal cords called bands of Büngner (Geuna et al. 2009). This process is also supported by other cells which contribute releasing diverse cytokines and neurotrophic factors, creating a new vascular network and synthesizing a pro-regenerative ECM (Nesbitt and Acland 1980; Mills 2007; Geuna et al. 2009; Carriel et al. 2014a).

Immediately after nerve transection, the *distal segment* starts a slow process of degeneration known as Wallerian degeneration (WD) (Rotshenker 2011). This process involves myelin sheath breakdown, SC proliferation, and immunological cell recruitment (mostly macrophages) (Geuna et al. 2009; Rotshenker 2011). It starts with the intrinsic degeneration of injured axons which triggers a complex multicellular response (Rotshenker 2011). Axonal membranes fuse and their ends seal while cytoplasmic components, like cytoskeleton, degenerate by proteolysis (granular disintegration) (Geuna et al. 2009). In parallel, the myelin sheaths retract from the RNs and become disrupted, forming globular structures. During this process, the SCs dedifferentiate, proliferate, and form bands of Büngner supporting nerve regeneration from the distal nerve stump. As myelin debris (especially myelin-related proteins) have an inhibitory effect on axonal regeneration processes, macrophage recruitment is key as they remove and recycle all these components (Stocum 2006; Chen et al. 2015b). Furthermore, macrophages can polarize to a pro-regenerative phenotype (M1) that contributes to axonal regeneration (Chen et al. 2015b). In case the microenvironment conditions are favorable, positive signs of nerve regeneration will be evident at the distal segment (such as abundant newly formed PNFs, different degrees of myelination, and adequate ECM reorganization). However, if prolonged denervation occurs a fibrotic response of the distal nerve stump will take place (Geuna et al. 2009).

The *interstump zone* is the area between the proximal and distal nerve stumps that advancing regeneration processes must cross to reinnervate the distal targets. Depending on the severity of the injury, this zone could be short or critical and the success of the whole nerve regeneration process will depend on what happens at this level (Lundborg 2004).

The vast research conducted in this field (mostly using nerve conduits) showed that the regeneration process along this area is organized in five well-known progressive phases: (1) fluid phase, (2) matrix phase, (3) the cellular migration phase, (4) the axonal phase, and (5) the myelination phase (Belkas et al. 2004; Lundborg 2004; Carriel et al. 2014a). The initial phase is characterized by the influx of plasma exudate from both nerve stumps being the trophic factors, cytokines, and ECM precursors essential to trigger the regeneration process. This is followed by the formation of a loose ECM, firstly composed of fibrin, which acts as a physical platform for cell migration throughout the gap. Cells, especially SCs, migrate from both nerve stumps establishing the bands of Büngner. With the advance of the cellular phase, the transitory fibrin clot is progressively replaced by a more stable ECM and this process is also accompanied by the formation of new blood vessels.

The establishment of this pro-regenerative stroma is determinant to initiate the axonal phase and adequately guide the axonal regeneration from the proximal nerve stump to the distal segments. Following axonal regeneration, SCs initiate the PNF maturation process resulting in the formation of functional myelinated and/or unmyelinated PNFs (Belkas et al. 2004; Lundborg 2004; Geuna et al. 2009; Carriel et al. 2011a; Carriel Araya 2017). The time needed for each phase to take place varies according to the animal model, the length and diameter of the defect, the graft or substitute used, and the conditions of the regenerative microenvironment (more details can be found in ► [“Appropriate Animal Models for Translational Nerve Research”](#) (Ronchi et al. 2020). From the experimental point of view, all phases are important (Gambarotta et al. 2013; El Soury and Gambarotta 2019; Gambarotta et al. 2019). However, in peripheral nerve tissue engineering, where the therapeutic efficacy of novel engineered substitutes are aimed, most functional and histological analyses are conducted during an active, incomplete, phase of axonal regeneration and/or myelination (Carriel et al. 2014c; Chato-Astrain et al. 2018, 2020).

3 Histological Techniques

As mentioned in the introduction, histological analyses are important and reliable quality controls in PN repair and regeneration studies (Vleggeert-Lankamp 2007; Raimondo et al. 2009; Carriel et al. 2014b). In this section the most relevant technical aspects related to the preparation of nerve samples for both light and electron microscopy are discussed.

Histological techniques or histotechnology are a series of technical procedures that allow analysis to be conducted by light (optical) or electron microscopy of cells, tissues, and organs (Carriel 2019). In the case of light microscopy, and based on the aims of the study, some tissues can be examined directly, such as the evaluation of m-PNFs by phase contrast microscopy (Kiernan 2007, Carriel et al. 2017a). However, significantly more reliable, accurate, and permanent results can be obtained on fixed, sectioned, and stained material for which a chronological order of technical procedures must be followed (Kiernan 2007; Di Scipio et al. 2008; Carriel et al. 2014b; Carriel 2019).

For light and electron microscopy, adequate tissue collection is essential. It is always recommended to conduct a proper, delicate, and relatively fast tissue dissection and harvesting. In nerve repair studies, gross examination could be carried out during dissection. This fast examination will provide useful information related to the nerve continuity and cable formation and even some adverse effects (fibrotic response and adhesions, inflammation, graft encapsulation, biomaterials degradation, rupture, collapse or rejection, etc.) which will be further confirmed by histology (Hernandez-Cortes et al. 2010; Chato-Astrain et al. 2018, 2020). Furthermore, special care should be taken during tissue harvesting, avoiding as much as possible hemorrhage, mechanical pressure, or traction of the tissue, especially at the areas of interest, which in the case of repaired nerves range from the proximal to distal nerve stumps.

Frequently, in these kinds of studies, the PN samples are accompanied by other elements of diverse chemical nature (biomaterials, suture material, particles, conduits, fluids, etc.) (see chapter “Biomaterials and Scaffolds for Repair of the Peripheral Nervous System”) (Carriel et al. 2014a). In general, natural and hydrophilic biomaterials (such as collagen, fibrin, chitosan, silk, acellular nerve grafts, etc.) are suitable for histological analyses (Belkas et al. 2004; Raimondo et al. 2005; Carriel et al. 2011a, 2013; Chato-Astrain et al. 2018; Lovati et al. 2018; Philips et al. 2018a). However, some elements could be difficult to evaluate by histological analyses and it is important to know in advance if they are suitable or not for light or electron microscopy studies. For example, synthetic, inorganic, metal-based, or hydrophobic biomaterials may cause technical problems due to their excessive hardness, high solubility in organic solvents (they will be dissolved during tissue processing), impermeability (they may affect the diffusion of the chemical agents), or they cannot be impregnated by inclusion media (paraffin or resin). In this scenario, it is advisable to test them before starting the experiments, and if they cause problems, it is convenient to remove them before those critical steps or at least after the fixation of the tissues (to ensure the structural stabilization of the cell and ECM components of interest).

Once the tissue sample is obtained, the tissue fixation and processing must be initiated immediately. For didactic reasons the technical procedures and applications of light and electron microscopy techniques are treated separately in this chapter.

3.1 Histological Techniques for Light Microscopy

In order to properly analyze the PN structure and regeneration process by light microscopy it is necessary to obtain sufficiently thin and transparent histological sections. For this purpose, the structure and chemical composition of the tissue must be stabilized through chemical and/or physical fixation. Moreover, an adequate tissue consistency must be obtained, for sectioning, which is achieved with the use of embedding media (Kiernan 2008; Carriel 2019). The physicochemical properties of PNs make them suitable material for both conventional paraffin embedding and crysectioning techniques (Kiernan 2007; Vleggeert-Lankamp 2007; Raimondo et al. 2009; Lovati et al. 2018; Carriel 2019).

3.1.1 Tissue Fixation and Paraffin Embedding

Nerve tissue histological processing starts with the fixation, which is considered the most critical step in histotechnology. Through diverse fixation techniques it is possible to stabilize the cellular and ECM components obtaining a closely comparable histoarchitecture to living conditions. Fixation also interrupts all metabolic and postmortem degradative processes (autolysis, apoptosis, and putrefaction) (Kiernan 2007, 2008; Carriel et al. 2017b). In the case of PN samples, and based on their chemical features, they are suitable for chemical, physical, and combined fixation techniques (Kiernan 2007; Di Scipio et al. 2008; Raimondo et al. 2009; Carriel et al. 2017b).

There are several chemical fixatives available which are classified as coagulant (alcohol-based agents), noncoagulant (additive agents), and combined (mixtures) (Kiernan 2008). Tissue must be immersed in at least 20 times its own volume of fixative solution. Moreover, better nerve histological patterns can be obtained in three different ways: i) nerve ends can be attached to a cork or similar material during fixation; ii) by adding a few drops of fixative to the nerve tissue prior dissection (in situ); and iii) by using an intracardiac perfusion technique (Kiernan 2008; Raimondo et al. 2009; Chato-Astrain et al. 2018, 2020).

Coagulant fixatives are not recommended for lipid or myelin analysis, as they will dissolve these elements (Kiernan 2008). Indeed, freezing and cryosectioning techniques are recommended for lipid histochemistry including myelin (Carriel et al. 2017b). Nevertheless, most lipids are unaffected by noncoagulant fixatives, especially formaldehyde, which induce stable methylene bond formation among tissue proteins. Fortunately, some myelin lipoproteins partially resist degradation by organic solvent enabling their histochemical identification in formaldehyde-fixed and paraffin-embedded samples (Kiernan 2007; Carriel et al. 2011a, 2014b, 2017a) where myelin usually appears as a reticular network around axons (artifact classically known as neurokeratin) (Fawcett and Jensch 1997; Carriel Araya 2017).

There are some specific fixatives for nerve tissue (Lillie's calcium acetate formalin or chromation-based fixation techniques) (Kiernan 2007), but 4% neutral buffered formaldehyde is preferred and the most used agent for this purpose because it is compatible with most histochemical and IHC procedures (Kiernan 2007; Carriel 2019). Another excellent fixative is osmium tetroxide (OsO_4). This reagent can be used as primary or more commonly as postfixation agent (after aldehyde-based fixatives) for light and especially electron microscopy (Kiernan 2007; Di Scipio et al. 2008; Raimondo et al. 2009). Osmium tetroxide (OsO_4) may react with proteins, and it is reduced by the lipids resulting in a black and insoluble precipitate which chemically stabilizes and stains (prior to embedding) these tissue components, including myelin. Osmicated tissues can be further analyzed with other histochemical and IHC techniques (Di Scipio et al. 2008; Raimondo et al. 2009; Carriel et al. 2017a) (Fig. 3).

In general, most authors immerse PN samples in 4% formaldehyde for a period ranging from 24–48 h at room temperature (Carriel et al. 2014c, 2017a, b). Tissues can be left longer in this agent, but epitopes will be affected for further IHC analyses (Kiernan 2008; Carriel et al. 2017a). Although the intracardiac perfusion with 4% formaldehyde or 4% paraformaldehyde is not really needed, this method offers the following advantages: i) results in a better fixation and overall histological pattern; ii) will prevent retraction of the nerve during dissection; iii) will greatly facilitate the gross examination and nerve dissection; and iv) will adequately fix the distal muscles for further morphometric and/or histological analyses (Carriel et al. 2013, 2014c; Chato-Astrain et al. 2018; Chato-Astrain et al. 2020). However, it will eliminate all blood elements from the blood vessels.

Once fixation is complete, it is important to define the orientation of the tissues for their subsequent processing, paraffin embedding, and analyses. In this sense, native and repaired nerves can be processed to obtain longitudinal or transverse histological

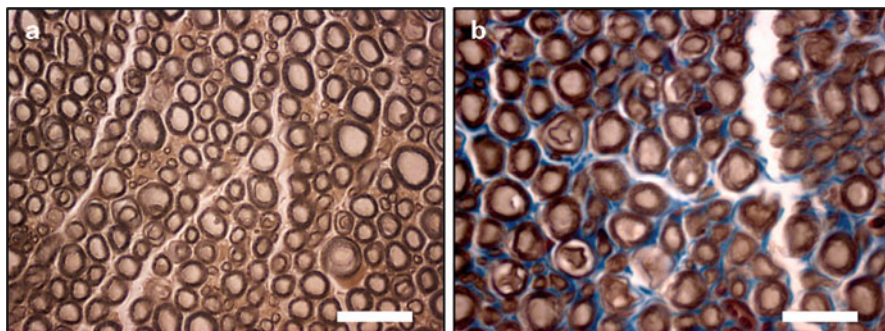


Fig. 3 Pre-paraffin embedding myelin stain with OsO_4 of a rat median nerve. In (a) a section without contrast is shown, while in (b) the histological section was contrasted with Masson's trichrome revealing the collagen content (blue reaction). Scale bar = $20\mu\text{m}$. Images were reproduced from Carriel et al. 2017a with permission from Springer

sections, both options being valid, informative, and widely used in the field (Carriel et al. 2013, 2014c; Chato-Astrain et al. 2018; Philips et al. 2018a) (Fig. 1). Fixed tissues are not suitable for direct paraffin infiltration and they must be completely dehydrated and cleared. Dehydration is conducted by replacing the water content in the tissue with an organic solvent, for which serial changes of ascending concentrations of ethanol are used (50° – 99°). Clearing procedures aim to equilibrate the tissue with an alcohol and paraffin miscible solvent, such as xylene or toluene. Both dehydrating and clearing solutions will dissolve most lipids, but as mentioned above, myelin will be partially preserved (Kiernan 2007). The paraffin waxes for histology are hydrophobic hydrocarbon polymers that provide hardness at room temperatures, but melt between 52 and 58°C . Cleared tissues are infiltrated with molten paraffin waxes and finally embedded in solid wax (Kiernan 2008; Carriel et al. 2017b; Carriel 2019). Small tissue samples are processed in few hours, whereas large, thick, and dense tissue samples will need longer time protocols (Kiernan 2008; Carriel et al. 2017b; Carriel 2019). The tissue processing (from dehydration to embedding) can be performed manually or by using an automatic tissue processor. Finally, the thickness of PN histological sections ranges from 5 to $7\mu\text{m}$.

3.1.2 Cryosectioning

This tissue processing protocol consists of the stabilization of the histological structure by means of freezing techniques. Freezing can be done directly on a fresh tissue (not recommended) or preferably after formaldehyde fixation. Direct freezing is useful for highly specific histochemical applications, such as the enzyme histochemistry of the intramural plexus in chronic constipation (Meier-Ruge and Bruder 2005), skeletal muscle enzyme histochemistry (Leiva-Cepas et al. 2020), or difficult immunofluorescence (IF) procedures (Cook and Warren 2015). However, this method results in a reversible structural and metabolic stabilization, and chemical postfixation techniques are frequently used (Kiernan 2008).

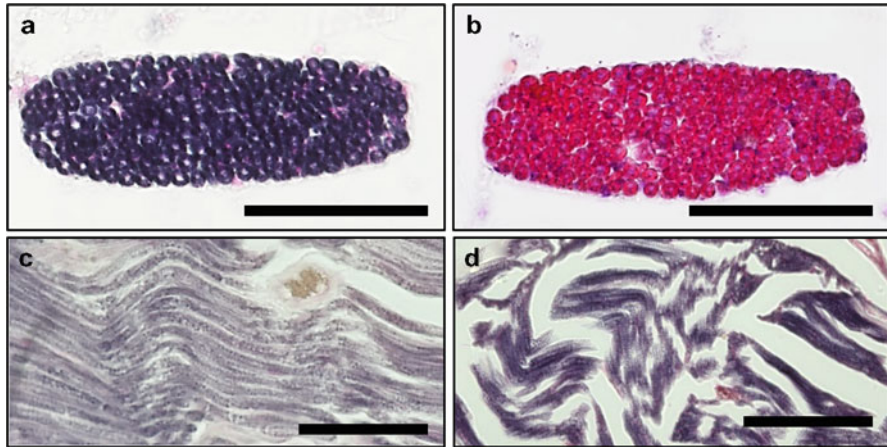


Fig. 4 Myelin staining on peripheral nerve cryosections. Images showing positive histochemical reactions of myelin in rat peripheral nerves. Sudan black B **a**), **c**), and **d**), and oil red O **b**) histochemical methods. Images **a–c**) were obtained from tissues subjected to formaldehyde fixation, sucrose cryoprotection, and fast freezing (-160°C), while the image **d**) shows structural damage due to the slow freezing procedure (-20°C) of a previously fixed, but not cryoprotected nerve. Scale bar = $100\mu\text{m}$ (**a** and **b**), $200\mu\text{m}$ (**c** and **d**). Images were reproduced from Carriel et al. 2017b with permission from Springer

Inadequate and especially slow freezing may result in the formation of ice crystals in the tissue with irreversible damage to the histoarchitecture. In order to avoid this artifact, the samples must be frozen quickly by using cooled isopentane [-80°C (freezer) or -160°C (liquid nitrogen)] (Carriel et al. 2017b; Leiva-Cepas et al. 2018). However, considerably better morphological results can be obtained if the samples are first fixed in 4% formaldehyde (24–48 hours), then cryoprotected with 20% sucrose (24 hours at 4°C), and finally frozen with cold isopentane (Carriel et al. 2017b). This technique is suitable for IHC and myelin histochemistry (Fig. 4), but not for enzyme histochemistry. Cryosection thickness ranges from $10\text{--}20\mu\text{m}$ and they must be used as soon as possible. Cryosections can be stored (between -25 and -80°C in sealed conditions) but just for a limited period of time (around 5 months) (Carriel et al. 2017b).

3.2 Technical Consideration for Electron Microscopy Analyses

Electron microscopes are useful tools which allow analysis at the subcellular level with a resolution limit of 0.25 nm (Carriel 2019). There are two types of electron microscopes: the scanning electron microscope (SEM) and the transmission electron microscope (TEM). SEM techniques are essential to evaluate the surface features of cells, tissues, or materials, being often used in the ex vivo ultrastructural characterization of engineered PN substitutes (Carriel et al. 2017c; Philips et al. 2018a; Garcia-Garcia et al. 2021), but its value in PN regeneration studies is limited. In

contrast, TEM and semithin sections are considered the gold standard techniques for the evaluation of PNFs (Raimondo et al. 2009; Ronchi et al. 2014; Lovati et al. 2018). For TEM analysis, samples must be small ($1 \times 1 \times 1$ mm) for adequate fixation, dehydration, and embedding. It is recommended to add some drops of fixative in situ to keep them straight, favor dissection, and ensure transverse orientation. Harvested samples are then immersed in a fixative, composed of 2.5% glutaraldehyde and 0.5% saccharose in 0.1 M Sørensen phosphate buffer (SPB, pH 7.4), and fixed between 4 and 8 h at 4 °C, rinsed and finally stored in SPB (Raimondo et al. 2009). Postfixation is conducted by immersion of the samples in 2% OsO₄ in SPB for 1 to 2 h (depending on the sample size and density) under agitation.

Fixed samples are dehydrated in ascending concentrations of ethanol (30°, 50°, 70°, 95°, and 99°). Times and passages vary among laboratories, but at least five passages of around 5 minutes each are recommended (Raimondo et al. 2009). Dehydrated samples are then infiltrated and embedded in a polymeric resin (epoxy, araldite, Glauert's mixture, etc.) which provide the optimal hardness for ultramicrotomy (semithin and ultrathin sections) (Ronchi et al. 2014; Carriel et al. 2017c; Carriel 2019).

Generally, semithin sections are used to check the tissue quality and to select the area of interest, but in PN studies they are one of the most valuable techniques (Geuna et al. 2009; Carriel et al. 2014b; Ronchi et al. 2014; Lovati et al. 2018). By using an ultramicrotome, sections ranging from 1–3 μm thickness are obtained, stained (1% toluidine blue in 1% borax) in a hot plate at 80 °C (30–45 s), rinsed in tap water (Raimondo et al. 2009; Ronchi et al. 2014), and finally analyzed with a light microscope. For TEM analysis, ultrathin sections (50–70 nm thickness) are placed on pioloform-coated grids, stained with a saturated solution of uranyl acetate and lead citrate for 15 and 7 min, respectively. The OsO₄ postfixation and heavy metal stainings confer the electrodense properties to some of the elements.

4 Histological Assessment of Peripheral Nerve Tissue Regeneration

Histological analyses are the most widely used quality controls in PN repair and regeneration studies (Vleggeert-Lankamp 2007; Geuna et al. 2009; Carriel et al. 2014b). Undoubtedly, the main objective of these analyses will be to adequately demonstrate the PN regeneration process in repaired PNFs. Histological assessment can be conducted on semithin sections stained with toluidine blue, TEM, chemically fixed and paraffin-embedded or frozen material. All available techniques are valid and useful, but semithin and TEM analyses are considered the gold standard techniques for this purpose (Raimondo et al. 2009; Ronchi et al. 2014; Sanen et al. 2017; Lovati et al. 2018; Dietzmeyer et al. 2020) and they will be discussed below.

Histology conducted on sections obtained from paraffin-embedded or frozen samples are also commonly used, especially when semithin or TEM analyses are not available. These techniques offer the valuable possibility of conducting, in the

same material, a wide range of histological stainings – routine stainings, histochemistry, metal reduction techniques, molecular-based staining (IHC and IF) – for the assessment of PN regeneration process or other highly relevant parameters such as the degree of tissue organization, the features of the host response, or even fate of the biomaterials used.

In the following sections histological staining for light microscopy (paraffin-embedded or frozen materials) and semithin and/or TEM analyses will be discussed separately.

4.1 Staining Methods for Light Microscopy

There are several staining techniques available to conduct a comprehensive histological assessment in PN repair and regeneration studies. In this chapter, some methods are selected and discussed according to their use in the histological assessment of the following key aspects: i) nerve tissue regeneration; ii) stromal regeneration and remodeling; iii) host tissue response; and iv) quantitative approaches in light microscopy. Furthermore, these methods as well as some additional markers are summarized in the Tables 1 and 2.

4.1.1 Assessment of Nerve Tissue Regeneration

The starting point of most histological analyses, conducted on histological sections from paraffin-embedded or frozen material, is the hematoxylin-eosin (HE) staining. This electropolar method is adequate for general examination of the nerve tissue histological pattern and host response. Nonetheless, HE is a nonspecific staining for many tissue elements (Raimondo et al. 2009; Carriel et al. 2011b; Philips et al. 2018b) and does not allow accurate assessment of the myelin content (Raimondo et al. 2009; Ronchi et al. 2014). Moreover, most studies are conducted during an active PN regeneration process where the identification of key elements, such as myelin, axons, and cell types, is considerably more difficult, making it necessary to employ more specific staining methods.

The degree of myelination obtained after PN repair varies according to several factors. Myelin demonstration is crucial as it undoubtedly confirms the following key aspects of the PN regeneration: i) the axonal regeneration occurred; ii) SCs change from a pro-regenerative to a functional phenotype; iii) m-PNFs are maturing and beginning to be functional; and iv) the whole regeneration process reaches the last phase of PN regeneration, the myelination (Di Scipio et al. 2008; Raimondo et al. 2009; Carriel et al. 2014b, c; Ronchi et al. 2014). Myelin is well preserved on frozen material, especially when it is fixed and cryoprotected, and its content can be evaluated with solvent dyes. These dyes stain lipid because they are more soluble in these compounds than in the solvents from which they are applied, and these methods are Sudan black B, Sudan IV, and oil red O (Fig. 4) (Kiernan 2007; Carriel et al. 2017a, b). Moreover, myelin can be stained by some fluorochromes, like FluoroMyelin Red which allows its identification in cryosection and fluorescence microscopy (Mohan et al. 2019). Myelin unlike other lipids can be identified on fixed and paraffin-embedded material, where it is partially

Table 1 Summary of the histological methods and immunodetection markers of the main nerve regeneration-related components

Application	Methods available
Nerve tissue regeneration	
General histological pattern	HE, trichrome stainings
Myelin sheath evaluation	Histochemistry: Sudan black B, Sudan IV, oil red O, LFB staining (Klüver-Barrera), and MCOLL (LFB myelin staining and PS collagen staining) Fluorochrome: FluoroMyelinRed Immunohistochemistry: P0, PMP22, MBP, and MAG Semithin sections: OsO ₄ postfixation and toluidine blue, Deprez's Bodian/LFB staining
Axonal regeneration	Structural proteins: Neurofilaments, tubulin subunits (mostly β -III tubulin), and peripherin Regeneration-related proteins: GAP-43 Other molecules: PGP 9.5 Metal reduction techniques: Bodian and Bielschowsky
Assessment of stromal regeneration and remodeling	
Fibrillar ECM components:	
Collagen network	Trichrome methods: (Masson, Gomori, Van Gieson, Mallory, Movat, Arteta, and Picrosirius) Immunohistochemistry: Collagen type I, III, IV, V, VI, XV, and others
Elastic fibers	Orcein, Verhoeff Weigert's elastin staining, aldehyde fuchsin Immunohistochemistry: Fibrillin, elastin
Reticular fibers	Gomori's silver impregnation technique, periodic acid-Schiff (PAS) histochemical method
Nonfibrillar ECM components	
Glycoproteins	Histochemistry: PAS Immunohistochemistry: Laminin and fibronectin
Proteoglycans	Histochemistry: Alcian blue and toluidine blue (metachromatic reaction in sulphated proteoglycans) Safranina O Immunohistochemistry: Decorin, versican, and fibromodulin
Basal membrane	Histochemistry: PAS, PS Metal reduction technique: Methenamine silver impregnation technique, Gomori's silver impregnation method Immunohistochemistry: Laminin, entactin/nidogen, and collagens (IV and VII)
Immunostaining of stromal and glial markers in peripheral nerve regeneration	
Schwann cells	Vimentin, CD56, CD57, and CD146, NCAM, p75, S-100, and myelin-related markers: P0, PMP22, MBP, and MAG
Remak cells	Vimentin, CD56, CD57, and CD146, NCAM, p75, S-100, and GFAP Negative for myelin-related markers
Perineurial cells	Vimentin, Occludins, ZO-1, CLDN1 CLDN3, tight junction proteins, cytokeratin 18, and EMA
Fibroblast cell lineage	Vimentin (fibroblast and myofibroblast), collagen type I and III, TE-7 (fibrocytes and fibroblasts), SMA, and desmin (myofibroblast)

(continued)

Table 1 (continued)

Application	Methods available
Vascular markers	Histochemistry: Collagen and basal membrane stainings Factor VIII, CD31, CD34, laminin, collagen IV, and SMA Lymphatic vessels: D2–40
Other immunohistochemical markers	
Cell proliferation	Ki-67, PCNA, cyclins, and histones
Cell migration	Syndecan-4, FAK, Phaxillin
Apoptosis	TUNEL method Immunohistochemistry: Caspases, such as 3

Table 2 Selection of some antigens for the characterization of immunological host response

Cell lineage	Antigen
T lymphocytes	CD2, CD3, CD4 (helper T cells), and CD8 (cytotoxic T cells)
NK lymphocytes	CD2, CD8, CD16, perforin, and granzyme
B lymphocytes	CD19, CD20, CD10 (pre-B cells), CD38 (activated B cells)
Plasma cells	CD31 reactive plasma cells CD38, CD138 syndecan-1, CD319, immunoglobulins
Monocytes	CD14, CD15, CD16, myeloperoxidase
Macrophages	CD68, CD74, CD163
M1 pro-inflammatory	CD 80, TNF α , IL 6, IL 12, iNOS
M2 pro-regenerative	CD23, CD163, CD206, TGF β , IL 10
Hematopoietic cells, except erythrocytes	CD 45 (leucocyte common antigen)
Mast cells	CD45, CD44, heparin, MCT (mast cell tryptase)
Neutrophils	CD66, collagenase, elastase
Eosinophils	Eosinophil cationic protein (ECP)
Basophils	Histamine, promajor basic protein (proMBP-1)

preserved. The classical method described by Klüver and Barrera (1953) combines Luxol fast blue (LFB) and cresyl violet dyes staining myelin in blue and mast cells in purple over a greenish background (Klüber and Barrera 1953; Carriel et al. 2011a, 2017a) (see example in Fig. 1b). This method is specific for myelin but unspecific for other PN tissue elements. In this context, the MCOLL histochemical method (from myelin and collagen staining) could provide better histological results. MCOLL combines LFB myelin stain with Picosirius (PS) collagen identification and hematoxylin nuclear contrast. This method allows the simultaneous evaluation of the histological pattern, the myelin, and collagen content in a single slide with high contrast and specificity (Carriel et al. 2011a, 2017a). This method was developed for the evaluation of PN regeneration, and since then it has been used for this purpose (Carriel et al. 2013; Cui et al. 2018; Chato-Astrain et al. 2018; Fertala et al. 2020). As previously mentioned, pre-embedding OsO₄ postfixation is an excellent alternative to ensure myelin

preservation and staining in paraffin-embedded samples. This procedure prevents myelin artifacts (swelling and extraction) and provides a specific, intense, and well-defined black-brown myelin stain, being a valuable tool for the identification of m-PNFs (Di Scipio et al. 2008; Raimondo et al. 2009; Carriel et al. 2017a; Schaakxs et al. 2017) (Fig. 3).

Myelin sheaths and m-PNFs can be specifically identified by immunostaining. Antibodies are available for several myelin-related proteins, such as P0, PMP22, MBP, or MAG (Kiernan 2007; Mills 2007; Philips et al. 2018b), and are often used in peripheral nerve regeneration studies (Hernandez-Cortes et al. 2014; Carnevale et al. 2018; Sun et al. 2018). Although these immunoreactions reveal the myelin location, these molecules can also be observed in the cytoplasm of myelin-forming SCs (or oligodendrocytes at the central level) even when myelin has not yet been fully formed (Kiernan 2007).

SCs are not only related to myelin formation, these cells are key elements during the whole regeneration process and therefore their presence represents a clear sign of active PN regeneration. As previously mentioned, activated pro-regenerative SCs migrate early from both nerve stumps forming guidance cues (bands of Büngner) for regenerating axons. SCs also produce ECM molecules and, at the end, they adopt a functional phenotype, which results in the establishment of newly formed m-PNFs and/or u-PNFs (Remak cells or Remak bundles) (Belkas et al. 2004; Geuna et al. 2009). The best option to identify these cells is by immunostaining. Myelinating SCs and Remak cells express a wide range of molecules, such as vimentin, some CD markers (56, 57, and 146), NCAM, protein p75, and S-100 protein. The last molecule is the most frequently used marker for SCs lineage. S-100 proteins regulate intracellular Ca^{++} homeostasis. There are several members which are localized in the nucleus and/or cytoplasm of SCs, Remak cells as well as other nervous and non-nervous cell types (Gonzalez-Martinez et al. 2003; Garcia-Martinez et al. 2017). It is difficult to differentiate SCs from Remak ones, as they share several markers and light microscopy does not really help. Myelinating SCs express all myelin-related proteins (mentioned above) while Remak cells do not, and tend to express GFAP (Mills 2007). In relation to vimentin, it is not a good marker for SC or Remak cells as this intermediate filament forms part of the cytoskeleton of the majority of mesoderm-derived cells, such as fibroblasts, immunological cells, vascular cells, muscle fibers, etc. (Mills 2007).

The demonstration of axonal regeneration along repaired nerves and grafted engineered substitutes is determinant (Fig. 5). Within a regenerative microenvironment, axons could be found at different stages of regeneration and/or maturation with or without signs of myelination or Remak bundle formation (Allodi et al. 2012; Chacon et al. 2012; Ducommun Priest et al. 2019). Therefore, the use of myelin or SCs stainings will not provide complete information about the heterogeneous population of axons present within the regenerative microenvironment (Carriel et al. 2014c). When axons are sectioned, their highly organized ultrastructure is altered and the surrounding microenvironment stimulates the growth cone formation (Allodi et al. 2012). This process mainly relies on cytoskeletal rearrangements (Kevenaar and Hoogenraad 2015) and three steps of growth cone advance are known: protrusion, engorgement, and consolidation (Mortimer et al. 2008). The process starts with

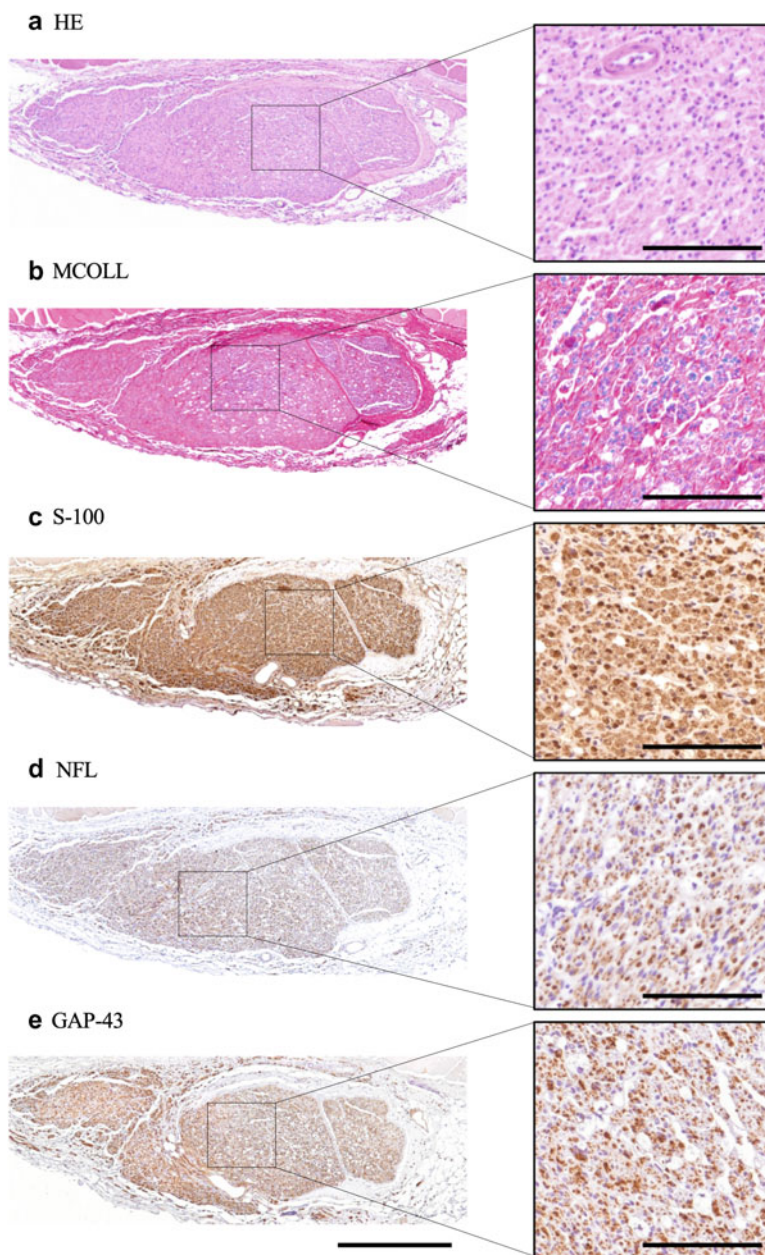


Fig. 5 Histology and distribution of histochemical and immunohistochemical positive reactions at the central portion of a nerve repair with autograft technique. In these transverse serial sections it is possible to identify the nerve tissue regeneration mostly at the endoneurial compartment level. MCOLL (B) denotes the collagen content of the stromal layers (red reaction) as well as some signs of myelination (blue reaction). Observe the abundant distribution of SCs (S-100) and regenerating axons (NFLs and GAP-43) as determined by immunohistochemistry (brown reaction). Scale bars = 500µm and 100µm (insets)

actin assembly followed by a progressive increase of tubulin isoforms (MTs) and peripherin (Allodi et al. 2012; Kevenaar and Hoogenraad 2015). NFLs are neuron-specific cytoskeletal proteins which are crucial for the definitive axonal structure. NFLs decrease in the proximal stump, but when the regenerating axon acquires certain structural stability, neurofilament cytoskeletal formation progressively occurs (Fig. 6) (Mortimer et al. 2008; Allodi et al. 2012). As expected during this complex process, several molecules (such as axonal transport proteins, adhesion molecules, cytoskeletal proteins, kinases, etc.) play specific roles from the beginning of axonal branching until axonal consolidation and myelination (Korshunova and Mosevitsky 2010; Allodi et al. 2012; Kevenaar and Hoogenraad 2015). GAP-43 or neuromodulin regulates cytoskeletal reorganization at the growth cone level during development (Korshunova and Mosevitsky 2010). This protein is relatively low under physiological conditions, but it is dramatically increased during PN regeneration where a differential expression with NFLs can be observe (Carriel et al. 2014c).

In order to identify regenerating axons, antibody-mediated detection can be used. Among all the markers, the identification of cytoskeletal proteins or those directly related to axonal growth are the most frequently used and recommended (Fig. 5). In this context, the identification of tubulin (mostly β -III tubulin) subunits will confirm MT formation, which is a clear indicator of axonal regeneration (Allodi et al. 2012; Carriel et al. 2014b, c). Similarly, the identification of NFLs (light, medium, and high molecular mass), which are responsible for radial axonal growth and thus determine the axonal diameter, are frequently used for the assessment of axonal regeneration by IHC (Fig. 5 D) and/or IF (Fig. 6) (Carriel et al. 2013, 2014c; Frost et al. 2018). The immunostaining of NFLs will allow the identification of axons during engorgement (structural maturation) and consolidation phases of axonal regeneration, but not immature ones (Allodi et al. 2012; Carriel et al. 2014c). In this sense, highly active regenerating and immature axons can be identified by GAP-43 immunostaining (Carriel et al. 2014c; Chato-Astrain et al. 2018). However, to perform a comprehensive histological assessment, active regenerating and maturing axons, both NFLs and GAP-43, should be used separately or by double immunostainings (Carriel et al.

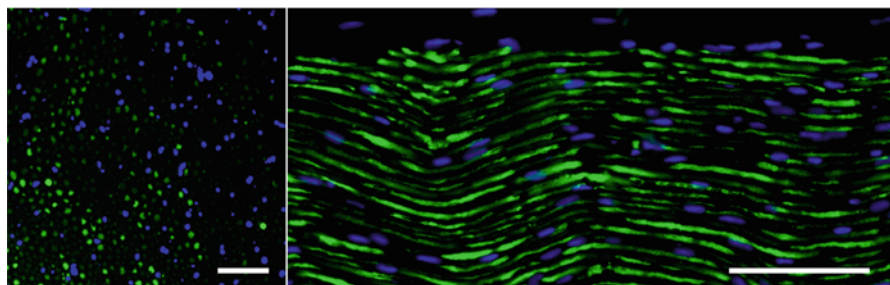


Fig. 6 Identification of neurofilaments by immunofluorescence microscopy. Note the intensity and distribution of medium- and high-molecular-mass NFLs (FITC, green) in a transverse and longitudinal section of a healthy rat sciatic nerve contrasted with DAPI (blue). Note the typical undulating course of nerve fibers in longitudinal sections. Scale bar = 100 μ m

2014b, c; Chato-Astrain et al. 2020). Peripherin, a type III neuronal intermediate filament, is also used for the assessment of axonal regeneration (Hol and Capetanaki 2017). This protein interacts with NFL subunits, and its immunoreactivity is restricted to peripheral neurons, central neurons that have peripheral axons, and predominantly unmyelinated axons (Hol and Capetanaki 2017).

Double immunostaining of peripherin and NFLs allow the simultaneous assessment of myelinated and unmyelinated PNFs (Fornaro et al. 2008). Another interesting protein for the immunostaining of axons is the protein gene product 9.5 (PGP 9.5) (Raimondo et al. 2009). It is a non-neuron-specific ubiquitin carboxyl-terminal hydrolase-1 which can be found in a wide range of healthy and cancerous cells (Campbell et al. 2003). Despite its unspecificity, this marker has been used in PN regeneration and reinnervation studies (Raimondo et al. 2009, Weng et al. 2018).

Finally, there are some classical metal reduction techniques for the assessment of neurofibrils (intracellular NFL networks). Methods and studies conducted by Santiago Ramón y Cajal described the axonal regeneration process more than 100 years ago (Ramón y Cajal et al. 1959; Yuste 2015). These methods were replaced by immunostainings, but there are two classical methods suitable for formalin-fixed and paraffin-embedded material, the Bodian and Bielschowsky silver impregnation techniques (Prophet 1992). These methods have been modified, combined, and compared over the years (Hightower and Gross 1985; Bolle et al. 1992) highlighting the modification described by Deprez (Deprez et al. 1999). In this work, authors combined Bodian's method with LFB myelin stain on semithin (resin-embedded) sections obtaining high-resolution images of both myelinated and unmyelinated PNFs (Deprez et al. 1999).

4.1.2 Assessment of Stromal Regeneration and Remodeling

The PN regeneration process involves remodeling and/or synthesis of new stromal layers. The process starts with ECM remodeling at both nerve stumps by the action of several matrix metalloproteases which creates a permissive microenvironment for cell migration and nerve tissue regeneration (Qin et al. 2016). Cells, mainly SCs and fibroblasts, migrate and produce a transitory ECM that bridges both nerve stumps. Classical histological methods are not able to specifically detect fibroblasts. For this reason, the expression of cellular markers, such as vimentin, needs to be evaluated through antibody-mediated recognition. More fibroblast markers are summarized in Table 1.

The ECM formed acts as a physical platform for the advance of nerve tissue regeneration, neovascularization, and substance diffusion (gas, cytokines, growth factors, etc.) (Belkas et al. 2004; Carriel et al. 2014a). With the advance and consolidation of the PN regeneration process, the newly formed ECM progressively acquired structural stability and organization (Belkas et al. 2004; Carriel et al. 2013). Some ECM molecules are essential for PN regeneration processes, as they regulate SC function. Pro-regenerative ECM molecules are some collagen fibers (types I, III, IV, V, VI, and XV) and the glycoproteins laminin and fibronectin (Chernousov et al. 2008; Chen et al. 2015a). Furthermore, the importance of the ECM molecules on PN regeneration is also well supported by the use of nerve grafts (auto and allografts),

acellular nerve grafts, and engineered substitutes, where natural ECM molecules or engineered scaffolds are used as guidance cues for the advancement of the growth cone (Carriel et al. 2014a; Georgiou et al. 2015; Lovati et al. 2018).

In general, the ECM is composed of diverse fibrillar proteins embedded in a polysaccharide-rich hydrated gel known as ground substance or nonfibrillar ECM (proteoglycans and glycoproteins) (Godoy-Guzman et al. 2018). The collagen, elastic, and reticular fibers are normal components of PN stromal layers (see details in Sect. 1.1.2) and there are some histological methods for their demonstration. In humans there are 28 proteins known as collagens, which are classified into several groups; the fibrillar collagens being the most abundant and suitable for histochemical identification. Most abundant fibrillar collagens can be stained by trichrome methods, such as Masson, Gomori, PS, Van Gieson, Mallory, or Arteta (Prophet 1992; Kiernan 2008; Cook and Warren 2015; Carriel 2019). These methods provide good contrast between nerve tissue and the surrounding collagen network allowing the correct assessment of the regenerating tissue organization pattern. Masson staining is often used in research, including PN regeneration studies (Alberti et al. 2016; Schaakxs et al. 2017; Qiao et al. 2019). In addition, PS method provides a well-defined and highly specific stain of collagens. The Sirius red F3B is a tetrakisazo dye, which when combined with picric acid (Picrosirius solution) binds to collagens and increases their natural birefringence allowing their organization pattern to be accurately determined under polarized microscopy (Carriel et al. 2011a, b) (Fig. 7 a, b). PS can be used alone, but more information can be obtained with the MCOLL histochemical method which, as mentioned above, specifically stains the myelin (Cui et al. 2018; Chato-Astrain et al. 2018; Fertala et al. 2020). Moreover, in order to describe the distribution of highly specific collagen fibers during nerve tissue regeneration immunostaining must be used (Evaristo-Mendonca et al. 2018; Philips et al. 2018b).

Regarding the elastic fibers, which are composed of elastin and diverse microfibrils, these can be identified by histochemical and immunostaining. Classical histochemical methods for elastic fibers are Orcein, Verhoeff, Weigert's elastin stain, or aldehyde fuchsin. These methods are easy, fast, economic, specific, and more sensible in comparison with the use of antibodies (Prophet 1992; Kiernan 2008). Elastic fibers are often evaluated in the ex vivo characterization of acellular nerve grafts (Philips et al. 2018a, b; Garcia-Garcia et al. 2021), but despite their important role in nerve biomechanics, their synthesis or remodeling during PN regeneration has not been deeply studied. Another important fiber type of the PN stroma are the reticular fibers, which form a thin structural network around the PNFs. Reticular fibers contain collagen type III and V, but also diverse polysaccharides and glycoproteins (Ushiki 2002), and they are commonly identified by Gomori's silver impregnation technique or periodic acid-Schiff (PAS) histochemical method (Philips et al. 2018b). In both procedures, the oxidation of the polysaccharides present in the molecule allows the identification of the fibers by silver solution or Schiff's reagent, respectively (Ushiki 2002; Kiernan 2008; Philips et al. 2018b).

For the identification of proteoglycans and glycoproteins, histochemical methods for carbohydrates and immunostaining are used. Proteoglycans represent a diverse

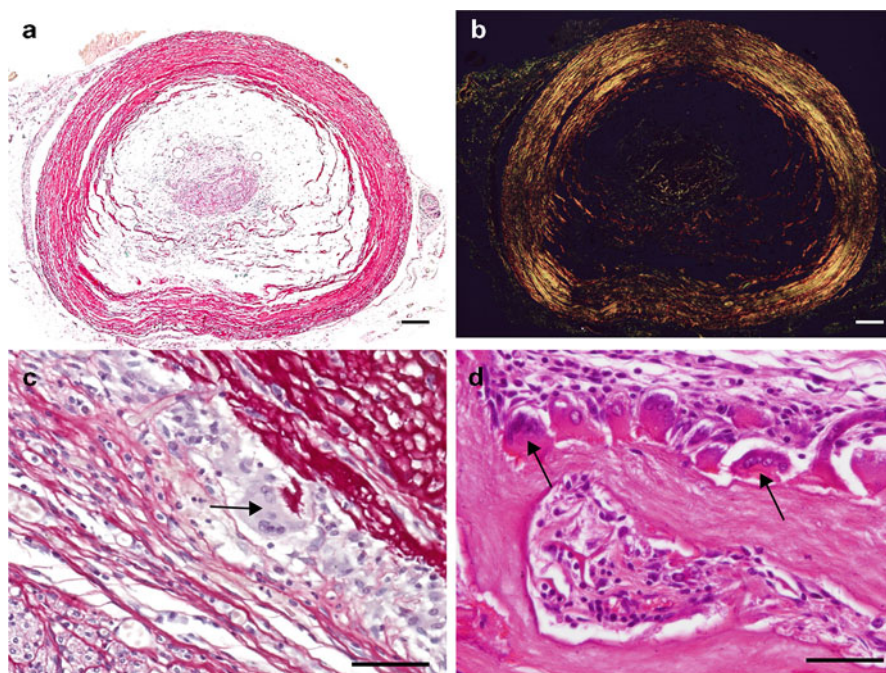


Fig. 7 Peripheral nerve regeneration and host response in a nerve repaired with a collagen-based nerve conduit. Transverse sections stained with PS histochemical methods viewed using light (a, c) and polarized microscopy (b), as well as HE method (d). Observe the intense histochemical reaction for the collagen which comprises the conduit. The high density and parallel organization of the collagen within the conduit results in an intense and specific birefringence (b). Figs. C and D show the typical organization and morphology of highly active foreign body reaction giant cell (arrows) at the nerve conduit surface where it is even possible to identify the biodegradation of the implanted biomaterial (arrow in C). Scale bars = 200µm (A and B) and 25µm (c and d)

group of molecules, which are difficult to classify (Iozzo and Schaefer 2015). Some proteoglycans (chondroitin sulfate proteoglycans) have an inhibitory effect on PN regeneration (Lovati et al. 2018; Garcia-Garcia et al. 2021), and thus their evaluation could help to understand the results obtained with the use of certain scaffolds or substitutes. For the identification of proteoglycans there are some methods available, being the Alcian blue and toluidine blue stainings the most frequently used (Garcia-Garcia et al. 2021). Alcian blue binds to carboxyl and sulphate ester groups of proteoglycans at pH 2.5, but only to the latter at pH 1 (Kiernan 2008). Toluidine blue also stains carbohydrates and in presence of sulphated groups a metachromatic reaction is observed; this method being a useful alternative to stain mast cell granules which contain heparin (Kiernan 2008). Glycoproteins are essential ECM molecules which regulate cell functions (adhesion, migration, and proliferation) and mediate cell-ECM interactions (Philips et al. 2018b). These molecules are distributed among the stromal layers, being also important components of the PNFs BM (Garcia-Garcia et al. 2021). Laminin is one of the most important, as it has the capability to promote,

sustain, and guide both axonal growth and SC functions (Gonzalez-Perez et al. 2013; Chato-Astrain et al. 2018; Lovati et al. 2018; Garcia-Garcia et al. 2021). Fibronectin is another connective tissue glycoprotein which plays similar roles to laminin during PN regeneration (Gonzalez-Perez et al. 2013). For this reason, the preservation of the endoneurial tubes, especially laminin after PN decellularization, is important (Philips et al. 2018b; Garcia-Garcia et al. 2021), and these facts also support their use in the functionalization of biomaterials for nerve repair (Cao et al. 2011; Gonzalez-Perez et al. 2013; Carriel et al. 2014a). During regeneration, the synthesis of laminin (BM formation) is determinant and precedes axon regeneration, and some authors used this parameter as an indicator of PN regeneration (Huang et al. 2017). An overview of the glycoprotein content and BM formation can be conducted with PAS, methenamine silver impregnation, or even PS methods (Philips et al. 2018b). However, the use of antibodies is by far the best and most accurate manner to specifically evaluate these components in light microscopy (Huang et al. 2017; Philips et al. 2018b).

The assessment of the ECM synthesis, remodeling, and proper organization in repaired PNs is important in order to confirm that both nerve stumps are connected by a mechanically stable and well-structured newly formed nerve tissue. Moreover, some histological staining could also be applied to evaluate the fate of the biomaterials used, as well as the host tissue response, which are discussed in following sections.

The stromal remodeling and PN regeneration also include different degrees of neovascularization of the regenerated segments (Farber et al. 2016; Lovati et al. 2018). The blood vessels are recognizable in histological sections stained with routine and/or histochemical techniques. Nevertheless, considerably more accurate results can be obtained by using antibodies against some vascular markers, such as the factor VIII, CD31, CD34, BM molecules (laminin or collagen IV), or smooth muscle actin (SMA) (Martín-Lacave and García Caballero 2012; Farber et al. 2016; Vela-Romera et al. 2019). The marker D2–40 can be employed for the identification of lymphatic vessel (Vela-Romera et al. 2019) (Table 1).

4.1.3 Assessment of Host Response

Detailed information about the host immunological response associated with nerve regeneration studies can be found in another chapter of this book (“The Immune Response and Implications for Nerve Repair”) and therefore only some key events, oriented to the histological assessment, will be discussed below.

Similar to the damage of other tissues and organs, the neural injury itself as well as its surgical repair causes cell, stromal, and vascularization damage. This activates the coagulation process (platelet activation, degranulation, and thrombus formation) and inflammatory response (Mikos et al. 1998). The process starts with an acute inflammatory response, which is mainly characterized by the recruitment of neutrophils, monocytes, and fibrin clot formation (Mikos et al. 1998; Campos et al. 2020). Immune activation of SCs regulates macrophage recruitment, which reabsorbs dead cells, ECM debris, and myelin. When foreign biodegradable biomaterials are used, these cells react, reabsorbing biomaterial particles and releasing proteolytic

enzymes. In the case of PN regeneration studies where long-term mechanical stability of biomaterials is needed, the inflammatory response may persist for weeks or several months (Chato-Astrain et al. 2018). The persistence of the biomaterials (and debris) often leads to a chronic inflammatory response or even foreign body reaction (Campos et al. 2021). Chronic inflammatory response is characterized by the recruitment of mononuclear leukocytes (lymphocytes, macrophages, and plasma cells) to the injury site or at the perivascular level (Chato-Astrain et al. 2018; Campos et al. 2020, 2021). Accompanying the regeneration process, it is possible to observe granulation tissue, which is composed of macrophages, fibroblasts, and myofibroblasts which contribute to the ECM remodeling, synthesis, and angiogenesis (Mikos et al. 1998). The foreign body reaction often occurs in slow-biodegradable biomaterials and it is characterized by the organization of macrophages and especially giant foreign body cells (multinuclear syncytial macrophages) around the biomaterials surface (Fig. 7c and d). Within a regenerative microenvironment the macrophages may adopt a pro-inflammatory (M1) or pro-regenerative (M2) phenotype (Cho et al. 2014; Murray et al. 2014). Furthermore, the inflammatory response to certain biomaterials may lead to different degrees of partial or complete fibrosis, such as the case of nonresorbable biomaterials, like silicon chambers.

Cells that constitute the acute and/or chronic inflammatory response can be easily identified in histological sections stained with HE or trichrome methods. In the case of macrophages and giant cells, they are characterized by their abundant and foamy cytoplasm, which could contain some visible particles or debris. Furthermore, the pathognomonic sign of giant cells is the presence of several cell nuclei (Mills 2007). Granulocytes are identified based on their size, nuclear morphology, and their well-known affinity for acid or basic dyes. In the case of mast cells, they are observed in routine staining, but they are specifically stained by some metachromatic histochemical reactions such as toluidine blue, cresyl violet, Giemsa, etc. (Kiernan 2008; Cook and Warren 2015). However, in order to conduct an accurate identification of these cells, several antibodies against surface molecules (cluster differentiation markers) must be used. More than 200 CD markers exist, and a selection of some markers is provided in the Table 2 (Martín-Lacave and García Caballero 2012; Cho et al. 2014; Murray et al. 2014). In addition, for the demonstration of adherences to the surrounding tissues following PN repair (Tos et al. 2016) or fibrotic host response (encapsulation) to implanted biomaterials or biomedical devices (such as nerve conduits), the histochemical methods for collagen fibers mentioned above can be employed with good results (Chato-Astrain et al. 2018; Campos et al. 2021).

4.1.4 Quantitative Approaches in Light Microscopy

Quantitative approaches are frequently applied to light microscopy images with the aim to quantitatively and objectively demonstrate key indicators of PN regeneration (Carriel et al. 2014b; Lovati et al. 2018). The information obtained with these procedures allows the existence of significant differences among experimental groups to be determined, which can be related to a better regeneration profile.

The quantitative assessment can be used for sections stained with routine, histochemical, and/or immunostainings (IHC and IF) (Carriel et al. 2014c). Currently, by using software for image analysis (such as Image J, Nis-Elements, or similar) it is possible to accurately determine the intensity and/or area fraction of the histochemical or IHC positive reactions (Carriel et al. 2011b, 2014c).

The evaluation of the intensity of a specific positive reaction may provide information related to the changes in the concentration of certain molecules. In nerve repair and regeneration studies, it was demonstrated that repaired nerves with acceptable regeneration profile have higher NFL intensity values than those with less tissue regeneration (Carriel et al. 2013, 2014c). In addition, repaired nerves exhibit less intensity for NFLs than native nerves used as controls, confirming their active regeneration and immature cytoskeleton (Carriel et al. 2013, 2014c; Cui et al. 2018; Chato-Astrain et al. 2020). Similarly, the intensity of myelin histochemical reaction or S-100 IHC were also related to better nerve tissue regeneration (Carriel et al. 2013; Chato-Astrain et al. 2020). In relation to the area fraction, this parameter is used to determine the percentage of area occupied by the positive reaction; it is also often referred as density. It was also demonstrated that area fraction values for histochemical (collagen and myelin) and IHC positive reactions (GAP-43, NFLs, and S-100) were related to the degree of nerve tissue regeneration (Carriel et al. 2013, 2014b, c). These parameters are not only useful for regeneration studies, indeed they were successfully applied in the quality control of nerve grafts generated by decellularization (Garcia-Garcia et al. 2021; ElSoury et al. 2021).

More recently, the length of NFLs and S-100 positive reactions were investigated as an indicator of PN regeneration. In this study, the authors demonstrated a relationship between longer (distally) positive reactions and better overall regeneration and functional recovery, which in this case was observed with the use of nerve autograft (Frost et al. 2018).

4.2 Assessment of Nerve Regeneration by Semithin and Transmission Electron Microscopy

The preparation of the nerve samples for TEM provides considerably better results than those obtained by light microscopy (Carriel et al. 2014b; Geuna 2015). The methodology used ensures an adequate nerve tissue fixation, myelin preservation (due to the OsO_4 postfixation), and the obtaining of transversally oriented semithin and ultrathin sections. Both alternatives allow a highly accurate, high-resolution, and quantitative analysis of the PN tissue regeneration to be conducted (Ronchi et al. 2014; Geuna 2015).

The analysis conducted on transverse semithin sections stained with toluidine blue provides high-resolution images of normal and regenerating m-PNFs (Ronchi et al. 2014) (Fig. 8). The classical indicators of nerve tissue regeneration are: i) the number of m-PNFs; ii) the density of m-PNFs; and iii) the m-PNFs-related diameters (Raimondo et al. 2009; Ronchi et al. 2014, Lovati et al. 2018).

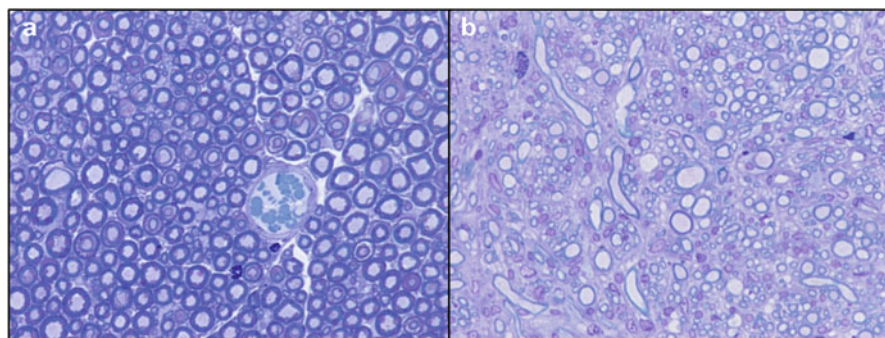


Fig. 8 Transverse semithin sections stained with toluidine blue. Histology of the endoneurial compartment of a healthy rat sciatic nerve (a) and a regenerating nerve fascicle inside the central portion of a collagen-based nerve conduit (b). Scale bar = 5 μm

Mean fiber density values are calculated by dividing the total number of nerve fibers within the sampling field by its area (N/mm^2). Regarding the total fiber number (N) it is estimated by multiplying the mean fiber density by the total cross-sectional area of the nerve (Raimondo et al. 2009). These values should be interpreted prudently, as high values of both parameters could be or not related to efficient and functional m-PNFs regeneration. Indeed, it was demonstrated that values from the proximal, interstump, and distal nerve stumps differed and must be correlated to functional parameters (Gordon 2009; Raimondo et al. 2009).

Furthermore, useful information can be obtained from m-PNFs size parameters. In this sense, it is possible to calculate the m-PNFs (D) and axon (d) diameters as well as the myelin sheath thickness $[(D-d)/2]$. Furthermore, by using the same data it is possible to obtain the g-ratio (D/d), all these parameters being related to the functional recovery of regenerating m-PNFs (Raimondo et al. 2009; Lovati et al. 2018).

Unfortunately, conventional semithin sections are not suitable for the accurate evaluation of u-PNFs. This limitation was solved by the technical modification described by Deprez et al. (Deprez et al. 1999). In this method, the samples are prepared for resin embedding, but omitting the use of OsO_4 . Furthermore, the resin is removed from the semithin sections and then stained with Bodian's impregnation technique followed by LFB myelin staining. This method allows the observation and quantitative assessment of myelinated and unmyelinated PNFs in light microscopy (Deprez et al. 1999).

Finally, TEM analysis permits high-resolution images of the ultrastructural features of the PNs to be obtained (Mills 2007; Geuna et al. 2009) (Fig. 9). By using TEM it is possible to perform accurate descriptive and quantitative analyses of both m-PNFs and u-PNFs, being even superior than semithin sections analyses (Ronchi et al. 2014). From the quantitative point of view, with this material it is possible to determine all the parameters described above. Besides, the high resolution of ultrathin section images offers the possibility to determine quantitatively the

Fig. 9 Transmission electron microscopy image of a regenerating peripheral nerve. The image corresponds to a regenerating fascicle composed of m-PNFs (black arrows) and u-PNFs (white arrows) along an acellular nerve graft in the rat sciatic nerve injury model.

SCs = Schwann cell nuclei and R = Remak cells nuclei. Scale bar = 5 μ m



unmyelinated/myelinated axon ratio, another valuable indicator of PN regeneration (Deprez et al. 1999; Lovati et al. 2018).

The high resolution of the TEM images also permits the accurate evaluation of other ultrastructural parameters, such as the presence of the PNFs BM, endoneurial collagen fibers organization, and the main ultrastructural features of blood vessel.

5 Conclusions

The peripheral nerve regeneration process is highly complex and needs to be studied comprehensively from the clinical, functional, and histological perspectives. The use of histological techniques to assess the peripheral nerve tissue regeneration are the most widely used quality controls in nerve repair and regeneration studies. The importance of the histological analyses in this field relies on the high specificity, sensitivity, and reliability of the results obtained, being the perfect complement of clinic-functional investigations.

In this regard, the main goal of histological analyses is to accurately demonstrate the peripheral nerve regeneration process after nerve repair, an adequate knowledge of nerve histology and its techniques being essential. Histology should not be limited to nerve regeneration assessment, because it also provides highly valuable and additional information concerning cellular, molecular, and/or pathological processes. Furthermore, light microscopy, as compared to semithin sections and electron microscopy, is characterized by its versatility. Indeed, there are several histochemical methods and antibodies available for the adequate assessment of

nerve regeneration and related processes. From the technical point of view, light microscopy studies are more simple and accessible than those conducted in semithin and transmission electron microscopy.

Semithin sections stained with toluidine blue and transmission electron microscopy analyses are considered the most accurate methods in the field, with considerably better resolution and results than light microscopy. Both methods are suitable to conduct morphometrical evaluations which provides a number of reliable and highly specific results, being more specific and detailed when conducted by transmission electron microscopy.

Finally, despite the usefulness and specificity of each technique separately, it is recommended to combine a panel of stainings and/or high-resolution images, to obtain a complete, specific, and accurate overview of the whole nerve tissue regeneration process.

6 Cross-References

- ▶ [Biomaterials and Scaffolds for Repair of the Peripheral Nervous System](#)
- ▶ [Surgical Techniques in Nerve Repair](#)
- ▶ [The Immune Response and Implications for Nerve Repair](#)

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References

- Alberti KA, Neufeld CI, Wang J, Xu Q (2016) In vivo peripheral nerve repair using tendon-derived nerve guidance conduits. *ACS Biomater Sci Eng* 2(6):937–945
- Allodi I, Udina E, Navarro X (2012) Specificity of peripheral nerve regeneration: interactions at the axon level. *Prog Neurobiol* 98(1):16–37
- Arroyo EJ, Scherer SS (2000) On the molecular architecture of myelinated fibers. *Histochem Cell Biol* 113(1):1–18
- Belkas JS, Shoichet MS, Midha R (2004) Peripheral nerve regeneration through guidance tubes. *Neurol Res* 26(2):151–160
- Bolle L, Maurer B, Janzer RC (1992) A modified Hortega-Globus stain is superior to Bielschowsky and Bodian stains for demonstrating neuritic plaques. *Biotech Histochem* 67(2):82–87

- Campbell LK, Thomas JR, Lamps LW, Smoller BR, Folpe AL (2003) Protein gene product 9.5 (PGP 9.5) is not a specific marker of neural and nerve sheath tumors: an immunohistochemical study of 95 mesenchymal neoplasms. *Mod Pathol* 16(10):963–969
- Campos F, Bonhome-Espinosa AB, Chato-Astrain J, Sanchez-Porras D, Garcia-Garcia OD, Carmona R, Lopez-Lopez MT, Alaminos M, Carriel V, Rodriguez IA (2020) Evaluation of fibrin-agarose tissue-like hydrogels biocompatibility for tissue engineering applications. *Front Bioeng Biotechnol* 8:596
- Campos F, Bonhome-Espinosa AB, Carmona R, Duran JDG, Kuzhir P, Alaminos M, Lopez-Lopez MT, Rodriguez IA, Carriel V (2021) In vivo time-course biocompatibility assessment of biomagnetic nanoparticles-based biomaterials for tissue engineering applications. *Mater Sci Eng C Mater Biol Appl* 118:111476
- Cao J, Sun C, Zhao H, Xiao Z, Chen B, Gao J, Zheng T, Wu W, Wu S, Wang J, Dai J (2011) The use of laminin modified linear ordered collagen scaffolds loaded with laminin-binding ciliary neurotrophic factor for sciatic nerve regeneration in rats. *Biomaterials* 32(16):3939–3948
- Carnevale G, Pisciotta A, Riccio M, Bertoni L, De Biasi S, Gibellini L, Zordani A, Cavallini GM, La Sala GB, Bruzzesi G, Ferrari A, Cossarizza A, de Pol A (2018) Human dental pulp stem cells expressing STRO-1, c-kit and CD34 markers in peripheral nerve regeneration. *J Tissue Eng Regen Med* 12(2):e774–e785
- Carriel V (2019) Métodos y técnicas de estudio en histología, embriología e ingeniería tisular bucodental. *Histología, Embriología e Ingeniería Tisular Bucodental*. E. Gómez de Ferraris and A. Campos, Editorial Panamericana: 14–36
- Carriel Araya VS (2017) Preclinical evaluation of bio-artificial conduits developed by tissue engineering for peripheral nerve regeneration Doctoral Thesis, Ghent University (Belgium); University of Granada (Spain)
- Carriel V, Garzon I, Alaminos M, Campos A (2011a) Evaluation of myelin sheath and collagen reorganization pattern in a model of peripheral nerve regeneration using an integrated histochemical approach. *Histochem Cell Biol* 136(6):709–717
- Carriel VS, Aneiros-Fernandez J, Arias-Santiago S, Garzon IJ, Alaminos M, Campos A (2011b) A novel histochemical method for a simultaneous staining of melanin and collagen fibers. *J Histochem Cytochem* 59(3):270–277
- Carriel V, Garrido-Gomez J, Hernandez-Cortes P, Garzon I, Garcia-Garcia S, Saez-Moreno JA, Del Carmen Sanchez-Quevedo M, Campos A, Alaminos M (2013) Combination of fibrin-agarose hydrogels and adipose-derived mesenchymal stem cells for peripheral nerve regeneration. *J Neural Eng* 10(2):026022
- Carriel V, Alaminos M, Garzon I, Campos A, Cornelissen M (2014a) Tissue engineering of the peripheral nervous system. *Expert Rev Neurother* 14(3):301–318
- Carriel V, Garzon I, Alaminos M, Cornelissen M (2014b) Histological assessment in peripheral nerve tissue engineering. *Neural Regen Res* 9(18):1657–1660
- Carriel V, Garzon I, Campos A, Cornelissen M, Alaminos M (2014c) Differential expression of GAP-43 and neurofilament during peripheral nerve regeneration through bio-artificial conduits. *J Tissue Eng Regen Med*
- Carriel V, Campos A, Alaminos M, Raimondo S, Geuna S (2017a) Staining methods for Normal and regenerative myelin in the nervous system. *Methods Mol Biol* 1560:207–218
- Carriel V, Campos F, Aneiros-Fernandez J, Kiernan JA (2017b) Tissue fixation and processing for the histological identification of lipids. *Methods Mol Biol* 1560:197–206
- Carriel V, Scionti G, Campos F, Roda O, Castro B, Cornelissen M, Garzon I, Alaminos M (2017c) In vitro characterization of a nanostructured fibrin agarose bio-artificial nerve substitute. *J Tissue Eng Regen Med* 11(5):1412–1426
- Chacon MR, Navarro AI, Cuesto G, del Pino I, Scott R, Morales M, Rico B (2012) Focal adhesion kinase regulates actin nucleation and neuronal filopodia formation during axonal growth. *Development* 139(17):3200–3210
- Chato-Astrain J, Campos F, Roda O, Miralles E, Durand-Herrera D, Saez-Moreno JA, Garcia-Garcia S, Alaminos M, Campos A, Carriel V (2018) In vivo evaluation of nanostructured fibrin-

- agarose hydrogels with mesenchymal stem cells for peripheral nerve repair. *Front Cell Neurosci* 12:501
- Chato-Astrain J, Philips C, Campos F, Durand-Herrera D, Garcia-Garcia OD, Roosens A, Alaminos M, Campos A, Carriel V (2020) Detergent-based decellularized peripheral nerve allografts: an in vivo preclinical study in the rat sciatic nerve injury model. *J Tissue Eng Regen Med* 14(6):789–806
- Chen P, Cescon M, Bonaldo P (2015a) The role of collagens in peripheral nerve myelination and function. *Mol Neurobiol* 52(1):216–225
- Chen P, Piao X, Bonaldo P (2015b) Role of macrophages in Wallerian degeneration and axonal regeneration after peripheral nerve injury. *Acta Neuropathol* 130(5):605–618
- Chernousov MA, Yu WM, Chen ZL, Carey DJ, Strickland S (2008) Regulation of Schwann cell function by the extracellular matrix. *Glia* 56(14):1498–1507
- Cho DI, Kim MR, Jeong HY, Jeong HC, Jeong MH, Yoon SH, Kim YS, Ahn Y (2014) Mesenchymal stem cells reciprocally regulate the M1/M2 balance in mouse bone marrow-derived macrophages. *Exp Mol Med* 46:e70
- Cook DJ, Warren PJ (2015) Cellular pathology: introduction to techniques and applications. Scion, Bloxham
- Cui Y, Yao Y, Zhao Y, Xiao Z, Cao Z, Han S, Li X, Huan Y, Pan J, Dai J (2018) Functional collagen conduits combined with human mesenchymal stem cells promote regeneration after sciatic nerve transection in dogs. *J Tissue Eng Regen Med* 12(5):1285–1296
- de Ruiter GC, Spinner RJ, Verhaagen J, Malessy MJ (2014) Misdirection and guidance of regenerating axons after experimental nerve injury and repair. *J Neurosurg* 120(2):493–501
- Deprez M, Ceuterick-de Groote C, Fumal A, Reznik M, Martin JJ (1999) A new combined bodian-luxol technique for staining unmyelinated axons in semithin, resin-embedded peripheral nerves: a comparison with electron microscopy. *Acta Neuropathol* 98(4):323–329
- Di Scipio F, Raimondo S, Tos P, Geuna S (2008) A simple protocol for paraffin-embedded myelin sheath staining with osmium tetroxide for light microscope observation. *Microsc Res Tech* 71(7):497–502
- Dietzmeyer N, Huang Z, Schuning T, Rochkind S, Almog M, Nevo Z, Lieke T, Kankowski S, Haastert-Talini K (2020) In vivo and in vitro evaluation of a novel hyaluronic acid-laminin hydrogel as luminal filler and carrier system for genetically engineered Schwann cells in critical gap length tubular peripheral nerve graft in rats. *Cell Transplant* 29:963689720910095
- Ducommun Priest M, Navarro MF, Bremer J, Granato M (2019) Dynein promotes sustained axonal growth and Schwann cell remodeling early during peripheral nerve regeneration. *PLoS Genet* 15(2):e1007982
- El Soury M, Gambarotta G (2019) Soluble neuregulin-1 (NRG1): a factor promoting peripheral nerve regeneration by affecting Schwann cell activity immediately after injury. *Neural Regen Res* 14(8):1374–1375
- El Soury M, García-García ÓD, Moretti M, Perroteau I, Raimondo S, Lovati AB, Carriel V (2021) Comparison of decellularization protocols to generate peripheral nerve grafts: A study on rat sciatic nerves. *Int J Mol Sci*. 2021 Feb 27;22(5):2389
- Evaristo-Mendonca F, Carrier-Ruiz A, de Siqueira-Santos R, Campos RMP, Rangel B, Kasai-Brunswick TH, Ribeiro-Resende VT (2018) Dual contribution of mesenchymal stem cells employed for tissue engineering of peripheral nerves: trophic activity and differentiation into connective-tissue cells. *Stem Cell Rev Rep* 14(2):200–212
- Farber SJ, Hoben GM, Hunter DA, Yan Y, Johnson PJ, Mackinnon SE, Wood MD (2016) Vascularization is delayed in long nerve constructs compared with nerve grafts. *Muscle Nerve* 54(2):319–321
- Fawcett DW, Jensch RP (1997) Bloom & Fawcett: concise histology. Chapman and Hall: International Thomson Pub, New York
- Fernandez R, Carriel V, Lage S, Garate J, Diez-Garcia J, Ochoa B, Castro B, Alaminos M, Fernandez JA (2016) Deciphering the lipid architecture of the rat sciatic nerve using imaging mass spectrometry. *ACS Chem Neurosci* 7(5):624–632

- Fertala J, Rivlin M, Wang ML, Beredjiklian PK, Steplewski A, Fertala A (2020) Collagen-rich deposit formation in the sciatic nerve after injury and surgical repair: a study of collagen-producing cells in a rabbit model. *Brain Behav* 10(10):e01802
- Fornaro M, Lee JM, Raimondo S, Nicolino S, Geuna S, Giacobini-Robecchi M (2008) Neuronal intermediate filament expression in rat dorsal root ganglia sensory neurons: an in vivo and in vitro study. *Neuroscience* 153(4):1153–1163
- Frost HK, Andersson T, Johansson S, Englund-Johansson U, Ekstrom P, Dahlin LB, Johansson F (2018) Electrospun nerve guide conduits have the potential to bridge peripheral nerve injuries in vivo. *Sci Rep* 8(1):16716
- Gambarotta G, Fregnan F, Gnani S, Perroteau I (2013) Neuregulin 1 role in Schwann cell regulation and potential applications to promote peripheral nerve regeneration. *Int Rev Neurobiol* 108: 223–256
- Gambarotta G, Raimondo S, Udina E, Phillips JB, Haastert-Talini K (2019) Editorial: peripheral nerve regeneration. *Front Cell Neurosci* 13:464
- Garcia-Garcia OD, El Soury M, Gonzalez-Quevedo D, Sanchez-Porras D, Chato-Astrain J, Campos F, Carriel V (2021) Histological, biomechanical, and biological properties of Genipin-crosslinked Decellularized peripheral nerves. *Int J Mol Sci* 22(2)
- Garcia-Martinez L, Campos F, Godoy-Guzman C, Del Carmen Sanchez-Quevedo M, Garzon I, Alaminos M, Campos A, Carriel V (2017) Encapsulation of human elastic cartilage-derived chondrocytes in nanostructured fibrin-agarose hydrogels. *Histochem Cell Biol* 147(1):83–95
- Gartner LP (2017) Textbook of histology. Elsevier, Philadelphia
- Georgiou M, Golding JP, Loughlin AJ, Kingham PJ, Phillips JB (2015) Engineered neural tissue with aligned, differentiated adipose-derived stem cells promotes peripheral nerve regeneration across a critical sized defect in rat sciatic nerve. *Biomaterials* 37:242–251
- Geuna S (2015) The sciatic nerve injury model in pre-clinical research. *J Neurosci Methods* 243: 39–46
- Geuna S, Raimondo S, Ronchi G, Di Scipio F, Tos P, Czaja K, Fornaro M (2009) Chapter 3: histology of the peripheral nerve and changes occurring during nerve regeneration. *Int Rev Neurobiol* 87:27–46
- Giorgadze T, Rukhadze R, Giorgadze S, Gujabidze N, Tevzadze N (2010) Quantitative changes of schwann and mast cells in the process of peripheral nerve regeneration. *Georgian Med News* (188):84–88
- Godoy-Guzman C, Nunez C, Orihuela P, Campos A, Carriel V (2018) Distribution of extracellular matrix molecules in human uterine tubes during the menstrual cycle: a histological and immunohistochemical analysis. *J Anat* 233(1):73–85
- Gonzalez-Martinez T, Perez-Pinera P, Diaz-Esnal B, Vega JA (2003) S-100 proteins in the human peripheral nervous system. *Microsc Res Tech* 60(6):633–638
- Gonzalez-Perez F, Udina E, Navarro X (2013) Extracellular matrix components in peripheral nerve regeneration. *Int Rev Neurobiol* 108:257–275
- Gordon T (2009) The role of neurotrophic factors in nerve regeneration. *Neurosurg Focus* 26(2):E3
- Griffin JW, Thompson WJ (2008) Biology and pathology of nonmyelinating Schwann cells. *Glia* 56 (14):1518–1531
- Hernandez-Cortes P, Garrido J, Camara M, Ravassa FO (2010) Failed digital nerve reconstruction by foreign body reaction to Neurolac nerve conduit. *Microsurgery* 30(5):414–416
- Hernandez-Cortes P, Toledo-Romero MA, Delgado M, Sanchez-Gonzalez CE, Martin F, Galindo-Moreno P, O'Valle F (2014) Peripheral nerve reconstruction with epsilon-caprolactone conduits seeded with vasoactive intestinal peptide gene-transfected mesenchymal stem cells in a rat model. *J Neural Eng* 11(4):046024
- Hightower M, Gross GW (1985) A combined Bodian-Nissl stain for improved network analysis in neuronal cell culture. *Stain Technol* 60(6):315–320
- Hol EM, Capetanaki Y (2017) Type III intermediate filaments Desmin, glial fibrillary acidic protein (GFAP), vimentin, and Peripherin. *Cold Spring Harb Perspect Biol* 9(12)

- Huang CW, Huang WC, Qiu X, da Silva FFF, Wang A, Patel S, Nesti LJ, Poo MM, Li S (2017) The differentiation stage of transplanted stem cells modulates nerve regeneration. *Sci Rep* 7(1): 17401
- Iozzo RV, Schaefer L (2015) Proteoglycan form and function: a comprehensive nomenclature of proteoglycans. *Matrix Biol* 42:11–55
- Junqueira LCU, Carneiro J (2005) Basic histology: text & atlas. McGraw-Hill Medical, New York
- Kevenaar JT, Hoogenraad CC (2015) The axonal cytoskeleton: from organization to function. *Front Mol Neurosci* 8:44
- Kiernan JA (2007) Histochemistry of staining methods for normal and degenerating myelin in the central and peripheral nervous systems. *J Histochemol* 30(2):87–106
- Kiernan JA (2008) Histological and histochemical methods: theory and practice. Scion, Bloxham
- Kierszenbaum AL (2019) Histology and cell biology : an introduction to pathology. St. Louis, Elsevier Inc
- Kierszenbaum AL, Tres LL (2016) Histology and cell biology: an introduction to pathology. Elsevier, Philadelphia
- Kluver H, Barrera E (1953) A method for the combined staining of cells and fibers in the nervous system. *J Neuropathol Exp Neurol* 12(4):400–403
- Korshunova I, Mosevitsky M (2010) Role of the growth-associated protein GAP-43 in NCAM-mediated neurite outgrowth. *Adv Exp Med Biol* 663:169–182
- Leiva-Cepas F, Ruz-Caracuel I, Peña-Toledo MA, Agüera-Vega A, Jimena I, Luque E, Peña J (2018) Metodología de laboratorio para el estudio histológico del músculo esquelético. *Arch Med Deporte*:254–262
- Leiva-Cepas F, Jimena I, Ruz-Caracuel I, Luque E, Villalba R, Pena-Amaro J (2020) Histology of skeletal muscle reconstructed by means of the implantation of autologous adipose tissue: an experimental study. *Histol Histopathol* 35(5):457–474
- Lovati AB, D'Arrigo D, Odella S, Tos P, Geuna S, Raimondo S (2018) Nerve repair using decellularized nerve grafts in rat models. A review of the literature. *Front Cell Neurosci* 12:427
- Lundborg G (2004) Nerve injury and repair. In: Regeneration, reconstruction and cortical re-modelling. Elsevier, Philadelphia
- Marettová E (2016) Expression of cytokeratin 18 in the peripheral nerves. *Folia Vet* 60(2):5–10
- Martín-Lacave I, García Caballero T (2012) Atlas de inmunohistoquímica [caracterización de células, tejidos y órganos normales. Madrid, Ediciones Díaz de Santos.; 1 online resource (413 páginas)
- Meier C, Dermietzel R, Davidson KG, Yasumura T, Rash JE (2004) Connexin32-containing gap junctions in Schwann cells at the internodal zone of partial myelin compaction and in Schmidt-Lanterman incisures. *J Neurosci* 24(13):3186–3198
- Meier-Ruge WA, Bruder E (2005) Pathology of chronic constipation in pediatric and adult coloproctology. *Pathobiology* 72(1–2):1–102
- Mescher A (2018) Junqueira's basic histology: text and atlas, 15th edn. McGraw-Hill Education
- Mikos AG, McIntire LV, Anderson JM, Babensee JE (1998) Host response to tissue engineered devices. *Adv Drug Deliv Rev* 33(1–2):111–139
- Mills SE (2007) Histology for pathologists. Lippincott Williams & Wilkins, Philadelphia
- Mills SE (2012) Histology for pathologists (Stacey E. Mills, ed). Philadelphia, Lippincott Williams & Wilkins
- Mohan S, Coto Hernandez I, Selig MK, Shibata S, Jowett N (2019) Stain-free resolution of unmyelinated axons in transgenic mice using fluorescence microscopy. *J Neuropathol Exp Neurol* 78(12):1178–1180
- Mortimer D, Fothergill T, Pujic Z, Richards LJ, Goodhill GJ (2008) Growth cone chemotaxis. *Trends Neurosci* 31(2):90–98
- Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdts S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T, Locati M, Mantovani A, Martinez FO, Mege JL, Mosser DM, Natoli G, Saeij JP, Schultze JL, Shirey KA, Sica A, Suttles J, Udalova I, van Ginderachter

- JA, Vogel SN, Wynn TA (2014) Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* 41(1):14–20
- Navarro X (2009) Chapter 27: neural plasticity after nerve injury and regeneration. *Int Rev Neurobiol* 87:483–505
- Nesbitt JA, Acland RD (1980) Histopathological changes following removal of the perineurium. *J Neurosurg* 53(2):233–238
- Oh SJ (2002) Color atlas of nerve biopsy pathology. CRC, Boca Raton
- Pavelka M, Roth J (2010) Functional ultrastructure: atlas of tissue biology and pathology. SpringerWein, New York
- Peltonen S, Alanne M, Peltonen J (2013) Barriers of the peripheral nerve. *Tissue Barriers* 1(3): e24956
- Philips C, Campos F, Roosens A, Sanchez-Quevedo MDC, Declercq H, Carriel V (2018a) Qualitative and quantitative evaluation of a novel detergent-based method for decellularization of peripheral nerves. *Ann Biomed Eng* 46(11):1921–1937
- Philips C, Cornelissen M, Carriel V (2018b) Evaluation methods as quality control in the generation of decellularized peripheral nerve allografts. *J Neural Eng* 15(2):021003
- Pina-Oviedo S, Del Valle L, Baquera-Heredia J, Ortiz-Hidalgo C (2009) Immunohistochemical characterization of Renaut bodies in superficial digital nerves: further evidence supporting their perineurial cell origin. *J Peripher Nerv Syst* 14(1):22–26
- Prophet EB (1992) Laboratory methods in histotechnology, Amer Registry of Pathology
- Qiao W, Lu L, Wu G, An X, Li D, Guo J (2019) DPSCs seeded in acellular nerve grafts processed by Myrolysin improve nerve regeneration. *J Biomater Appl* 33(6):819–833
- Qin J, Zha GB, Yu J, Zhang HH, Yi S (2016) Differential temporal expression of matrix metalloproteinases following sciatic nerve crush. *Neural Regen Res* 11(7):1165–1171
- Raimondo S, Nicolino S, Tos P, Battiston B, Giacobini-Robecchi MG, Perroteau I, Geuna S (2005) Schwann cell behavior after nerve repair by means of tissue-engineered muscle-vein combined guides. *J Comp Neurol* 489(2):249–259
- Raimondo S, Fornaro M, Di Scipio F, Ronchi G, Giacobini-Robecchi MG, Geuna S (2009) Methods and protocols in peripheral nerve regeneration experimental research: part II—morphological techniques. *Int Rev Neurobiol* 87:81–103
- Ramón y Cajal S, May RM, National Institute on Drug Abuse and Addiction Research Center (U.S.) (1959) Degeneration & regeneration of the nervous system. Hafner Pub, New York
- Rodriguez FJ, Folpe AL, Giannini C, Perry A (2012) Pathology of peripheral nerve sheath tumors: diagnostic overview and update on selected diagnostic problems. *Acta Neuropathol* 123(3): 295–319
- Ronchi G, Jager SB, Vaegter CB, Raimondo S, Giacobini-Robecchi MG, Geuna S (2014) Discrepancies in quantitative assessment of normal and regenerated peripheral nerve fibers between light and electron microscopy. *J Peripher Nerv Syst* 19(3):224–233
- Ronchi G, Gambarotta G, Morano M, Fregnan F, Pugliese P, Tos P, Geuna S, Haastert-Talini K (2020) Critical analysis of the value of the rabbit median nerve model for biomedical research on peripheral nerve grafts. *J Tissue Eng Regen Med* 14(5):736–740
- Rotshenker S (2011) Wallerian degeneration: the innate-immune response to traumatic nerve injury. *J Neuroinflammation* 8:109
- Sanen K, Martens W, Georgiou M, Ameloot M, Lambrichts I, Phillips J (2017) Engineered neural tissue with Schwann cell differentiated human dental pulp stem cells: potential for peripheral nerve repair? *J Tissue Eng Regen Med* 11(12):3362–3372
- Schaakxs D, Kalbermatten DF, Pralong E, Raffoul W, Wiberg M, Kingham PJ (2017) Poly-3-hydroxybutyrate strips seeded with regenerative cells are effective promoters of peripheral nerve repair. *J Tissue Eng Regen Med* 11(3):812–821
- Spiegel I, Peles E (2002) Cellular junctions of myelinated nerves (review). *Mol Membr Biol* 19(2): 95–101
- Stocum DL (2006) Regenerative biology and medicine. Elsevier Academic Press, Amsterdam/Boston

- Sun Q, Tu H, Xing GG, Han JS, Wan Y (2005) Ectopic discharges from injured nerve fibers are highly correlated with tactile allodynia only in early, but not late, stage in rats with spinal nerve ligation. *Exp Neurol* 191(1):128–136
- Sun X, Zhu Y, Yin HY, Guo ZY, Xu F, Xiao B, Jiang WL, Guo WM, Meng HY, Lu SB, Wang Y, Peng J (2018) Differentiation of adipose-derived stem cells into Schwann cell-like cells through intermittent induction: potential advantage of cellular transient memory function. *Stem Cell Res Ther* 9(1):133
- Thorsen F, Rosberg HE, Steen Carlsson K, Dahlin LB (2012) Digital nerve injuries: epidemiology, results, costs, and impact on daily life. *J Plast Surg Hand Surg* 46(3–4):184–190
- Topp KS, Boyd BS (2006) Structure and biomechanics of peripheral nerves: nerve responses to physical stresses and implications for physical therapist practice. *Phys Ther* 86(1):92–109
- Tos P, Crosio A, Pellegatta I, Valdatta L, Pascal D, Geuna S, Cherubino M (2016) Efficacy of anti-adhesion gel of carboxymethylcellulose with polyethylene oxide on peripheral nerve: experimental results on a mouse model. *Muscle Nerve* 53(2):304–309
- Ubogu EE (2013) The molecular and biophysical characterization of the human blood-nerve barrier: current concepts. *J Vasc Res* 50(4):289–303
- Ushiki T (2002) Collagen fibers, reticular fibers and elastic fibers. A comprehensive understanding from a morphological viewpoint. *Arch Histol Cytol* 65(2):109–126
- Vela-Romera A, Carriel V, Martin-Piedra MA, Aneiros-Fernandez J, Campos F, Chato-Astrain J, Prados-Olleta N, Campos A, Alaminos M, Garzon I (2019) Characterization of the human ridged and non-ridged skin: a comprehensive histological, histochemical and immunohistochemical analysis. *Histochem Cell Biol* 151(1):57–73
- Vleggeert-Lankamp CL (2007) The role of evaluation methods in the assessment of peripheral nerve regeneration through synthetic conduits: a systematic review. *Laboratory investigation. J Neurosurg* 107(6):1168–1189
- Welsch U, Sobotta J (2008) *Histología*. Editorial Médica Panamericana, Madrid
- Welsch U, Sobotta J, Negrete JH (2009) *Sobotta Welsch histología*. Editorial Médica Panamericana, Madrid
- Weng YL, Wang X, An R, Cassin J, Vissers C, Liu Y, Liu Y, Xu T, Wang X, Wong SZH, Joseph J, Dore LC, Dong Q, Zheng W, Jin P, Wu H, Shen B, Zhuang X, He C, Liu K, Song H, Ming GL (2018) Epitranscriptomic m(6)a regulation of axon regeneration in the adult mammalian nervous system. *Neuron* 97(2):313–325.e316
- Yuste R (2015) From the neuron doctrine to neural networks. *Nat Rev Neurosci* 16(8):487–497



Mathematical Modeling for Nerve Repair Research

Simão Laranjeira, Rachel Coy, and Rebecca J. Shipley

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Abstract

Peripheral nerve tissue engineering has great promise for growing replacement tissues to treat peripheral nerve injuries. To date, the design of replacement tissues has focused on in vitro and in vivo experiments to characterize and test the impact of a vast range of materials, cells, and other factors, with limited translation to human

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trials. Here we propose and discuss the use of *in silico* modeling as a complementary approach to inform and accelerate the design of engineered replacement tissues.

Mathematical modeling in nerve regeneration is a small research field; however, there is a rich literature in using mathematical modeling to describe a host of highly relevant underpinning mechanisms (such as neurite growth, chemical diffusion, and angiogenesis) in different contexts. These models broadly fall into three categories: continuum (or averaged), discrete (or individual), and hybrid approaches, which combine the two. The key features and potential of these modeling categories are presented alongside a decision tree for matching a specific biological problem to the appropriate modeling approach. To be predictive, it is essential that mathematical models are benchmarked against experimental data. We present tools to efficiently fit models to such data (optimization) and investigate the reliability of model predictions (sensitivity analysis). Finally, this work concludes with two demonstrative case studies on the use of mathematical modeling (one continuum, one discrete) to tackle biological and design questions in peripheral nerve injury repair.

1 Introduction

1.1 Background

Peripheral nerves enable cross-talk between the central nervous system and peripheral organs, via neuronal communication along sensory and motor fibers. They are comprised of nerve fascicles (a bundle of axons along which electrical signals propagate), blood vessels, and support materials wrapped in a protective connective tissue layer (the epineurium); within a fascicle, axons and small blood vessels are embedded in a highly aligned matrix referred to as the endoneurium. Axons are wrapped in Schwann cells to provide trophic support, whereas blood vessels supply nutrients by diffusion for metabolic cell processes.

Peripheral nerve injuries (PNIs) occur when this architecture is disrupted, either through a nerve crush or transection, resulting in the disruption of the entire nerve physiology. Unlike the majority of tissues in adult mammals, the peripheral nervous system has an intrinsic capacity for repair and regeneration following injury. Immediately following an injury, a cascade of complex and coupled biological events are initiated involving both neuronal and non-neuronal supportive cells and signaling molecules. After transection or a crush-type injury, the axons and myelin in the distal stump of the severed nerve degenerate quickly, usually beginning within 24–36 h of the injury, through a process called Wallerian degeneration (Wang et al. 2015; Zochodne 2008).

Around 24–48 h after an injury, Schwann cell (SC) proliferation increases in both the proximal and distal stumps of the injured nerve and SCs migrate into the nerve gap to clear the axonal and myelin debris created by Wallerian degeneration, aided by macrophages. The SCs also shed their myelin sheaths and undergo an important change in phenotype to a repair Büngner phenotype (Jessen and Mirsky 2016). This is marked by an increase in proliferation rate, and the secretion of factors that promote neuronal and vascular growth and support the survival of existing neurons (Napoli et al. 2012; Wang et al. 2015), including brain-derived

neurotrophic factor, glial cell line-derived neurotrophic factor (GDNF), nerve growth factor (NGF), and vascular endothelial growth factor (VEGF) (Boyd and Gordon 2003; Fontana et al. 2012; Frostick et al. 1998; Jessen and Mirsky 2016). Repair-type SCs are also able to remodel the extracellular matrix (ECM) via the production of basement membrane proteins such as laminin and fibronectin.

If a crush injury has taken place, the endoneurium remains intact. If transection has occurred, a bridge-like structure develops across the nerve gap (Cattin et al. 2015; Jurecka et al. 1975). The repair-type SCs align within the endoneurial tubes to form bands of Büngner, and elongating endothelial cell columns and sprouting axons extend into the nerve gap using these bands as guidance (Parrinello et al. 2010). Regeneration in crush-type PNIs is generally more successful than in transection-type injuries due to the preservation of original nerve structures, which serve as a template for repairing axons and blood vessels and facilitate reinnervation (Nguyen et al. 2002), and therefore surgical intervention is more likely for transection-type injuries.

The regenerative processes for a transection-type injury are summarized in Fig. 1.

The simplest surgical technique for a transection-type injury is suturing the two stumps together; however, this may induce non-physiological tension and a sub-optimal repair. Clinical best practice for PNIs with a gap >3 cm is to bridge the injury site with a graft taken from the patient (an autograft, Fig. 2), providing structural support and physiological cues to encourage regeneration. However, removing a section of healthy nerve from the patient can cause complications such as loss of function and neuroma formation at the donor site, exacerbated if multiple grafts are required for more complex injuries. Furthermore, Grinsell and Keating (2014) report that only approximately 50% of autograft patients achieve functional recovery. This motivates the development of alternative approaches to match or even surpass the therapeutic efficacy of nerve autografts.

Advances in tissue engineering offer promising opportunities to build nerve repair constructs (NRCs) that could serve as alternatives to autografts. NRCs are generally cylindrical in shape to mimic the geometry of the damaged nerve and aim at directing and maximizing nerve regeneration across a nerve gap induced by injury. Due to the biological complexity of neuronal regeneration, NRC design requires careful consideration of multiple components (Muheremu and Ao 2015; Patel et al. 2018; Dodla et al. 2019): (1) scaffold/substrate, (2) growth factors, (3) extracellular matrix, and (4) therapeutic cells. An NRC design may combine a subset of these components, or all of them, as summarized in Fig. 3. This has stimulated extensive research on NRC design and testing (using both *in vitro* and *in vivo* models), spanning a large number of scaffold materials, seeded cells, and combinations thereof, with varying degrees of success (Deumens et al. 2010; Nectow et al. 2012). Base scaffold materials range from synthetic polymers to biopolymers such as Type I collagen and fibrin; indeed Angius et al. (2012) report on at least 70 different material substrates for NRCs that have been tested in animal models. For a detailed analysis of the current state-of-the-art on NRC scaffolds please see the corresponding chapters in this book. Additional structural components such as aligned rods or channels and porous surfaces have been explored to increase cell guidance cues (Daly et al. 2012). Although trials of many different NRC designs have offered some promising results, the efficacy of an NRC is yet to surpass that of an autograft in a

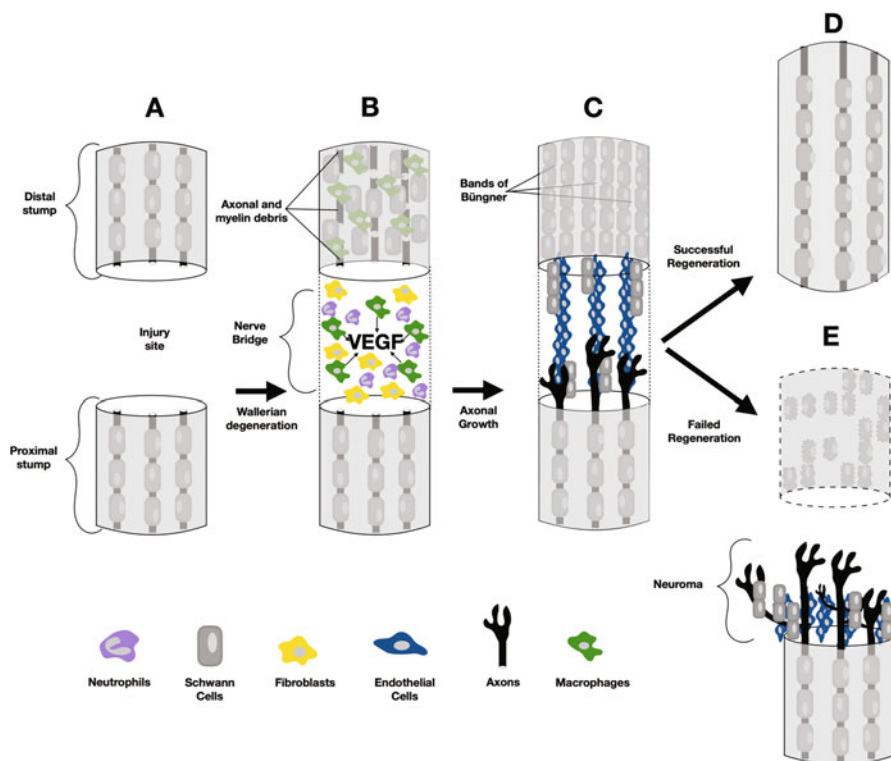


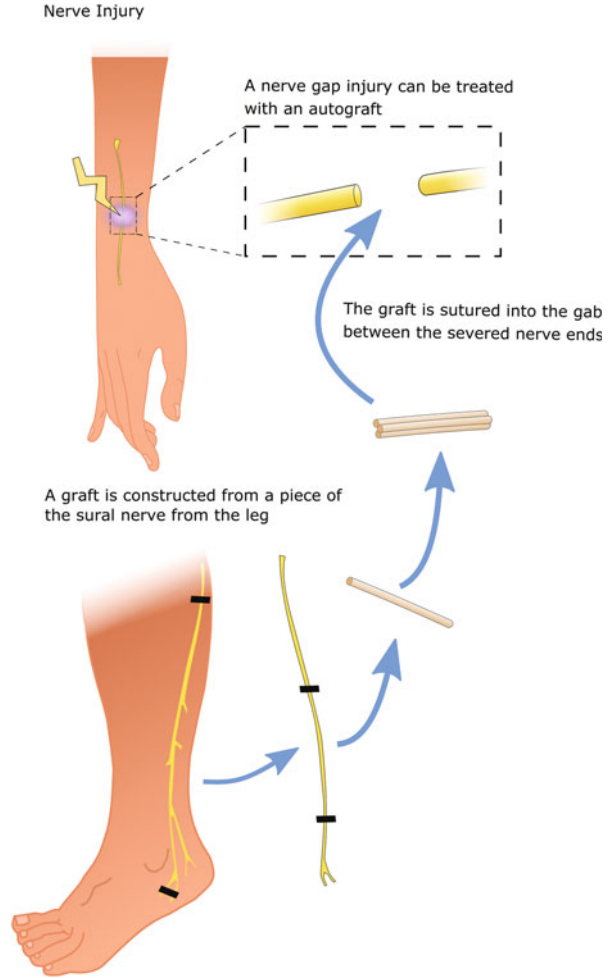
Fig. 1 Summary of the regenerative processes stimulated by a transection-type injury, adapted from the work by Chung (2018). (a) A transection injury leads to the formation of two nerve stumps, proximal and distal, separated by the injury site. (b) Wallerian degeneration is triggered, marked by the formation of a nerve bridge comprised of inflammatory cells such as macrophages and neutrophils, as well as fibroblasts and endothelial cells. At the distal stump, macrophages clear axonal and myelin debris. (c) Schwann cells migrate across the bridge from both stumps and form aligned structures called the bands of Büngner. Endothelial cell columns and sprouting axons follow, guided by the physical and chemical environment in the bridge. (d) Regeneration occurs if axons are able to cross the gap and reconnect with the distal stump. (e) If this is not possible, regeneration is interrupted, a neuroma may form due to the concentration of disconnected sprouting neurites, and the distal stump continuum to degenerate

clinical setting, indicating that further research is still required (Faroni et al. 2015; Gu et al. 2014; Lundborg 2003; Nectow et al. 2012).

1.2 The Role of Mathematical Modeling

Mathematical modeling has significant potential to provide a systematic approach to exploring the vast range of NRC options, informing focused experimental evaluations and accelerating new NRC designs toward the clinic. Such mathematical models must be parameterized and validated against dedicated experimental data

Fig. 2 Schematic (adapted from “Peripheral nerve graft – Mayo Clinic”) of PNI repair through nerve autograft surgery. If there is a gap in the nerve tissue following injury then this can be bridged using a section of nerve from elsewhere in the patient (autograft), for example, the sural nerve in the leg



in order for them to be predictive, and this necessitates close collaboration between mathematical and experimental teams. Essentially, validated mathematical models of the nerve repair process can be used to conduct computer-based experiments to explore how different NRC designs impact on the rate and success of nerve regeneration. The outputs of these computational experiments will enable much more focused evaluations of those NRC designs that offer the highest chance of success, reducing the number of animal experiments, cost, and time to move through preclinical toward clinical evaluations compared to a solely experimental approach. In contrast to experiments, once a mathematical model has been developed and validated, new simulations to test different designs are comparatively quick and very cheap to run. Furthermore, mathematical models can be used to generate hypotheses,

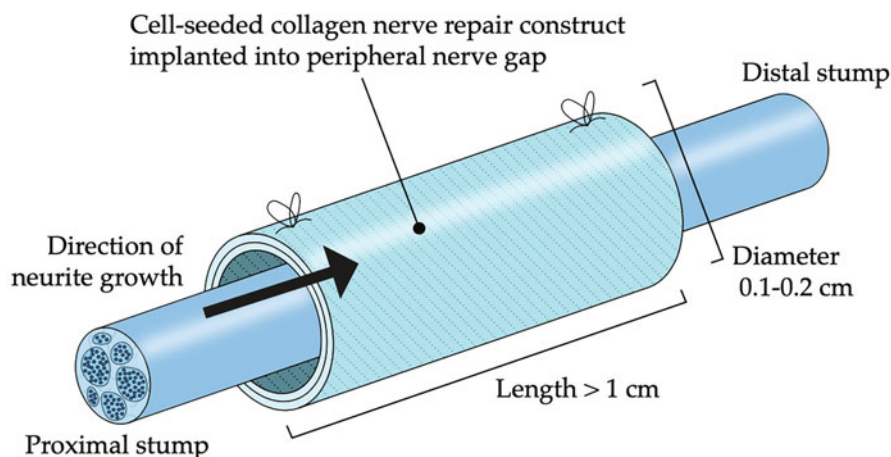


Fig. 3 Schematic of an NRC sutured between the proximal and distal nerve stumps. The body of the NRC can comprise a variety of components, including extracellular matrix materials, scaffolds, seeded cells, and growth factors (Coy 2020)

as well as test them. Sweeping over different parameter values in the computational models has the potential to unearth unexpected and exciting results that can inspire future experimental research.

Furthermore, mathematical models could help to bridge the species-specific gap between animal and human studies. Rat models feature in a majority of *in vivo* peripheral nerve repair studies, but frequently the results do not translate smoothly to human clinical application (Angius et al. 2012; Kaplan et al. 2015). In the future, mathematical models of peripheral nerve regeneration (PNR) could incorporate features and parameters relating specifically to human biology, which might help us to understand the differences between the rat model and the human more thoroughly.

Despite this potential, mathematical modeling remains a relatively new approach to PNI repair, although it is much more established in other areas of biology and medicine including to describe relevant regenerative mechanisms (e.g., chemotaxis-driven angiogenesis, durotaxis, etc.). In Sect. 2 we present these established modeling frameworks and their potential for application to PNI.

Finally, we conclude with two case studies on the use of mathematical modeling in the context of nerve regeneration; first the optimization of seeded therapeutic cell density to generate angiogenic cues, and second the growth of neurites within an NRC.

2 Mathematical Frameworks

Mathematical modeling in biology and medicine is a mature research field, and a multitude of mathematical models describing tissue growth and regeneration already exist. These models demonstrate a range of different approaches to describe biological behaviors that are underpinned by common biochemical and biomechanical

features and provide an extensive platform upon which to build similar approaches for peripheral nerve tissue engineering. For example, cell-solute interaction models have been developed and implemented to inform the design of tissue engineering bioreactors (Burova et al. 2019; O'Dea et al. 2012), tumor growth has been the subject of a vast number of computational studies, typically with a focus on tumor angiogenesis, biomechanics, or the interactions between tumor cells and nutrients or signaling factors (Metzcar et al. 2019). Most existing frameworks can be categorized as either continuum (averaged) or discrete (individual). Next, the theoretical background behind these frameworks is presented and strategies for combining discrete and continuum models are presented.

2.1 Continuum Frameworks

Reaction-diffusion-advection and mechanistic continuum models are commonly used for modeling spatiotemporal changes in concentrations of one or more species, at an averaged or population scale (for example, tracking cell density, rather than individual cells). For example, the change in concentration of a solute (C) over time, can be written as a partial differential equation (PDE), where the concentration is determined by the balance of diffusion (with coefficient D), convection by a velocity field ($\vec{v}$), and a reaction term (R) that incorporates consumption, production, degradation, or binding of the solute, summarized as:

$$\frac{\partial C(x, y, z, t)}{\partial t} = D(x, y, z, t) \nabla^2 C(x, y, z, t) - \vec{v}(x, y, z, t) \cdot \nabla C(x, y, z, t) + R(x, y, z, t), \quad (1)$$

where t is time, x , y , and z are the 3D spatial coordinates and ∇^2 is the Laplacian operator. Such models have been extended and applied to describe a range of biological processes including vascular development (Boas et al. 2018; Metzcar et al. 2019), tumor growth and response to therapy (Breward et al. 2002; Burova et al. 2019; Ward and King 1997), cellular motility and proliferation (Croll et al. 2005; Lewis et al. 2005). Functional (or constitutive) relationships are used in the models to define hypotheses around how different variables depend on each other (for example, Michaelis-Menten kinetics is often used to describe the relationship between cellular oxygen metabolism and the underlying oxygen availability and cell density, and would be used to describe the term $R(x, y, z, t)$ in Eq. (1)). Therefore, hypotheses can be tested by altering the functional forms, adding new terms or carrying out parameter sensitivity analyses to determine the impact on the predictions of the model. With such a mathematical modeling framework it is also possible to explore the role of different experimentally defined features on NRC outcomes, for example, different cell seeding densities and spatial distributions, or nutrient supply conditions.

Two continuum models of nerve regeneration have already been developed. First of all, in 1995 Podhajsky and Myers published a mathematical model of nerve regeneration that simulated neurite and blood vessel growth, Schwann cell proliferation, Wallerian degeneration, and fibrin matrix growth as reaction-diffusion processes. Two scenarios were simulated using this single generalized model: a nerve transection injury, where regeneration occurs from the proximal stump to the distal stump, and a nerve crush injury, where regeneration progresses from an area with no Wallerian degeneration toward a distal region with Wallerian degeneration. A combination of previously gathered experimental data and additional *in vivo* experiments were used to refine the model.

Instead of explicitly modeling the effects of oxygen deprivation (such as hypoxia) and growth factors on neurite and blood vessel growth, in this model SC proliferation is dependent upon the ratio of SCs to vessels (representing access to nutrients), and the ratio of degenerating tissue to SCs (representing the relative quantity of growth factors released by the tissue). Model predictions qualitatively mimicked the experimentally observed progression of regeneration, and predicted a traveling wave of vasculature, which precedes neuronal growth. This motivates further investigation into inducing faster, directional vascular growth by adapting NRC design.

The Podhajsky and Myers (1995) model uses many dimensionless coefficients, identified using literature values and curve fitting to available experimental data from a range of different models and setups, rather than dedicated experiments. Furthermore, the authors use dimensionless variables throughout, making it challenging to relate quantitative predictions to measures that could be validated experimentally.

A second model of nerve regeneration was published by Rutkowski and Heath in 2002 and consisted of a reaction-diffusion model of nutrient and nerve growth factor transport within a hollow porous tubular NRC (Rutkowski and Heath 2002). In this model, SCs seeded on the internal surface of the NRC lumen produce nerve growth factor, which diffuses both into the lumen and out of the construct and is consumed by neuronal cells. Both the SC and neuronal cell populations consume oxygen and glucose, and the diffusion of oxygen and glucose into the NRC into the lumen from the outside “bulk” material is also included. The long-time (steady state) behavior of the model was solved, rather than exploring the transition to that steady state.

The impact of changing the thickness and porosity of the NRC wall was investigated by running simulations with different values for these parameters. The model predicted that the concentration of nerve growth factor within the NRC lumen is increased when porosity is low and the wall is relatively thick, but that the same porosity values also result in a decrease in the oxygen concentration within the lumen which is undesirable for axon growth.

In vitro experiments were conducted alongside the theoretical work to derive many of the model parameters, such as nutrient diffusion coefficients, nutrient consumption rates by SCs and neurons and the nerve growth factor-dependent axon growth rate. In a second paper, model predictions were compared to measures of axonal growth recorded from the use of NRCs with varying porosity and wall

thickness configurations in vitro (Rutkowski and Heath 2002). The authors' hypotheses about the impact of wall porosity and thickness qualitatively agreed with the experimental results. However, the predicted values of oxygen and nerve growth factor were not directly validated as the experimental data recorded only axonal growth metrics.

One of the advantages of simple continuum models is the opportunity to readily interpret the link between the model and the importance of underpinning mechanisms. Nondimensionalization, where all variables are scaled related to typical values, can be used to inspect the relative sizes of reaction-diffusion model parameters and this can lead to simplification of the model as small terms are neglected (for example, metabolism terms may be neglected if they are shown to be negligible compared to diffusion). This makes the models simpler and faster to solve, and offers the opportunity to better understand the balance of biological processes and mechanisms that give rise to observable behaviors.

2.2 Discrete Models

An alternative approach to continuum modeling is the use of discrete models, which simulate behavior at the scale of individual cells or agents. Agent-based models simulate the actions and interactions of a population of autonomous agents, such as animals or cells, via a series of rules that dictate how the agents behave and interact with each other and their environment (Azuaje 2011). They have the capacity to incorporate a large number of rules that can track each cell agent independently with individual parameters, enabling the heterogeneity of behavior in biological systems to be captured. Additionally, these algorithms are nondeterministic or stochastic (as the rules that govern the agents can be probabilistic), therefore, they can allow for the natural variability or noise expected in these kinds of biological systems (Metzcar et al. 2019). As a consequence, these discrete models enable predictions to be made with incomplete knowledge of the biological phenomena being investigated.

In order to illustrate an agent-based modeling approach, we next describe an illustrative example of a discrete model for cells, growth factors (GF), and antagonists (A), and their interactions (see Fig. 4). These entities occupy a $n \times m$ grid (Fig. 4A) where, initially, there is a distribution of GFs and As across the grid domain. To capture a specific seeding scenario, cells are initially placed at the bottom of the grid. In each iteration of the algorithm, the states of all the cells and the chemical factors are updated, which consist in cells moving or migrating to a neighbor with a certain probability (P) dependent on the presence/absence of GF and A. The factors in turn diffuse within the grid, and consumption and/ or production by both cells and the environment can also be included via functional relationships. The neighborhood of the cells in a 2D grid is defined by the single-site spaces surrounding the cell (Fig. 4B). If two cells encounter each other (Fig. 4C)

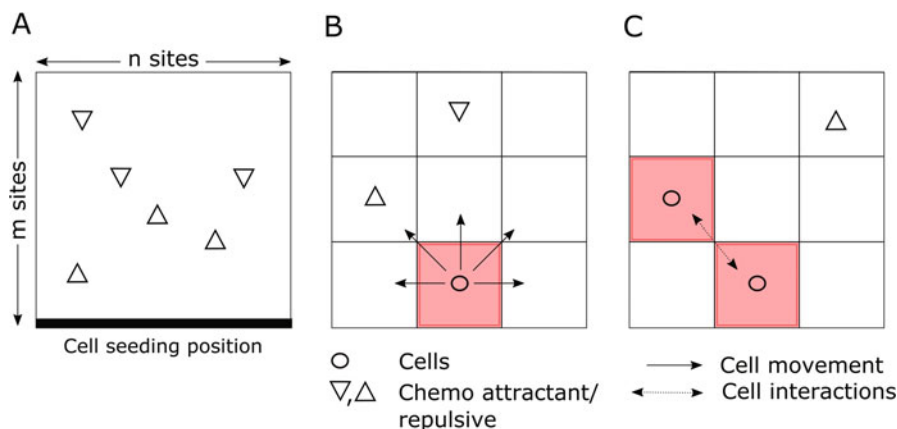


Fig. 4 The basic concepts of an agent-based model: (A) the spatial domain is represented by an $n \times m$ element grid, where a cell can reside in an individual element, with an underlying distribution of growth factor (GF) and antagonist (A) across the whole domain; (B) at each iteration, each cell has a probability of moving to any of the neighboring grid points, which is informed by GF and A concentrations at that grid point; (C) cell interactions are described explicitly in the rule framework

their interactions are defined by explicit functions (for example, in the case of endothelial cell models, this can also include the formation of networks (McDougall et al. 2002)). Iterations of the algorithm are run until a stopping criterion is reached (e.g., a defined time period), and the cell population, GF and A distributions through space and time are outputted from the simulation.

The agent-based model described in Fig. 4 belongs to a family of discrete models referred to as *lattice-based models*, where cells are tracked relative to a rigid grid (or spatial domain). Conversely, *off-lattice models* do not require such restrictions. Next, these two strategies are illustrated by comparing an example of each of a lattice-based and off-lattice approach: the Cellular Potts Model (CPM) and the Langevin equation, respectively. The models presented are not specific to nerve regeneration (as these approaches have not yet been applied in this context), however, they have been used more broadly to describe the growth of, and interaction between, cell populations relevant to PNR.

The CPM uses several lattice sites (or elements) to represent each cell, thereby allowing the definition of cell motility as well as cell connectivity and shape. Taking the example in Fig. 4 once more, the CPM algorithm looks at the $n \times m$ grid as a system with a certain energy, which can be interpreted as the cumulative balance of forces of attraction or repulsion being exerted on the cells. If a system starts with cells distant from each other to the point where they do not detect or interact with each other, and there are no chemotactic cues present, the system is at a steady state, and only changes occur stochastically. Alternatively, if a force is applied to it, the system will try to reach a steady state again through an iterative process that tries to minimize the energy of the whole system. The process by which it achieves that is

described by Hirashima et al. (2017), as, for each time step, the CPM algorithm iterates over all the pixels (2D) or voxels (3D) of the grid and performs random swaps (P_r) with neighboring pixels/voxels. For each target swap, the net energy of the system (ΔH) is calculated as the difference between the energy of the system before the swap (H_{before}) and after (H_{after}). If the energy decreases, i.e., $\Delta H \leq 0$, the swap is accepted in a deterministic way. Alternatively, if the energy increases ($\Delta H > 0$) the swap is rejected or accepted based on a stochastic Boltzmann function as:

$$P_r = \begin{cases} 1 & \text{if } \Delta H \leq 0 \\ \exp(-\Delta H/T) & \text{if } \Delta H > 0 \end{cases} \quad (2)$$

The parameter T is the Boltzmann temperature that determines the likelihood of a random change being accepted (Metzcar et al. 2019), can be interpreted as the sensitivity of a cell to the forces or cues acting on it and can be adapted for every case-specific implementation. Tissue location, geometry, and area are regulated within the model to favor stronger bonds between cells, so that the volume occupied by cells increases under favorable conditions.

The global energy is calculated following a Hamiltonian function (H), which accounts for each cell $\sigma(x) \in \{0, \dots, q\}$ where x is the coordinates of each lattice element and q is the number of individual cells simulated. The Hamilton function has three terms, firstly, a term, firstly, a term J defines the interaction between each cell $\sigma(x)$ and its neighbors $\sigma(x')$. Secondly, cellular shape and volume are defined via a relationship that relates the current area/volume of the cell $a(\sigma)$ with target area $a_{\text{tat}}(\sigma)$. The third term accounts for the sum of any other forces $F(\sigma)$ acting on the system. The standard formulation of H is summarized by:

$$H = \sum_{\text{neighboring sites}} J[\tau(\sigma(x)), \tau(\sigma(x'))][1 - \delta(\sigma(x), \sigma(x'))] + \lambda_A \sum_{\sigma} [a(\sigma) - a_{\text{tat}}(\sigma)]^2 + \sum F(\sigma), \quad (3)$$

where τ accounts for cellular type, $\delta(x, y)$ is a delta function that is 1 when $x = y$ and 0 otherwise. And λ_A encodes cell resistance to compression. The procedure of this algorithm is summarised in Fig. 5.

Similar formulations have been implemented in numerous contexts to explore cellular behaviors. For example, Allena et al. (2016) use this approach to simulate 3D cellular migration in the presence of durotactic cues, where the terms in Eq. (3) are adapted to account for cell adhesion and specific cell volume, surface area features. The J term, i.e., the interaction between neighboring cells, is interpreted as a function that characterizes the cells' bias to expanding toward neighbors with higher adhesion bonds. The second term is expanded to account for the impact of cellular surface area and volume on compression. The Boltzmann temperature is here interpreted as intrinsic cell motility.

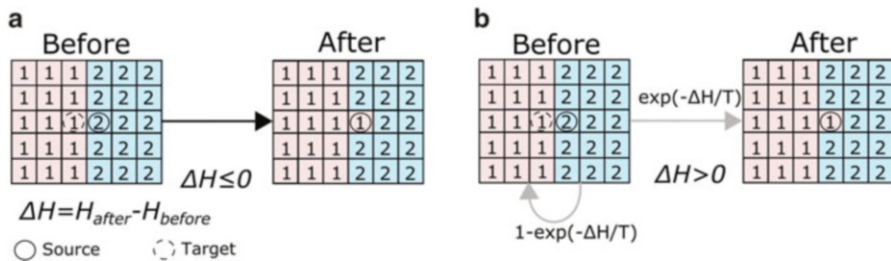


Fig. 5 Schematic representation, adapted from Hirashima et al. (2017), of the basic principles of a Cellular Potts Model (CPM). For each iteration of the model a target swap to the system is chosen at random, and difference between the current energy of system and the proposed change is evaluated (ΔH), following Eq. (3). If the energy of the system decreases, the swap is performed (A). Conversely (B), if there is an increase in energy the change can occur with the probability $e^{-\frac{\Delta H}{T}}$, where T is the Boltzmann temperature

The Allena et al. (2016) model was parameterized by identifying relevant ranges of parameters from the literature, such as the initial cell volume and cell surface area. However, a limitation of discrete models is here illustrated, as the majority of parameters have to be estimated through a sensitivity analysis methodology – there is not a wealth of reliable, quantitative cell-scale data from experiments available to benchmark these models. Despite this, the model was shown to reasonably predict the morphology of the cell, the distance covered, the spread area, and the migration speed when compared to experimental data.

Although the discrete components of the CPM can capture a variety of cellular behavior, some biomedical features benefit from a continuum modeling approach; for example, the diffusion of chemicals is commonly described using partial differential equations. This has motivated the development of hybrid approaches where a continuum model of a chemical field is coupled to the discrete CPM, for example, capturing chemotaxis by biasing cell movement in the direction of higher or lower chemical concentration (Boas et al. 2018). A typical formulation consists in adapting the term $\sum F(\sigma)$ from Eq. 3 to include a chemotaxis function (ΔH_{chem}), defined as:

$$\Delta H_{chem} = \lambda_{chem}(c(x) - c(x')), \quad (4)$$

where c is the chemical field and λ_{chem} is the sensitivity of the cells to the chemical gradient field.

In the literature, numerous models have utilized such a framework to model angiogenesis (Boas et al. 2018). Here we focus on the approach of Merks et al. (2006) as an example of a comprehensive hybrid modeling approach, which has also been validated against data from in vitro experiments. The model aims to explain the self-organization of endothelial cells and the formation of capillary-like structures in wound healing. They explore the interaction of mechanisms responsible for cell remodeling such as active cytoskeleton processes, and the response of endothelial cells to gradients in vascular endothelial growth factor (VEGF) concentration,

produced from a single source. A continuum model of the VEGF concentration (c) captures diffusion, the secretion of chemical cues by cells and degradation through

$$\frac{\partial c}{\partial t} = \alpha(1 - \delta(\sigma(x), 0)) - \varepsilon\delta(\sigma(x), 0)c + D\nabla^2 c, \quad (5)$$

where α is the secretion rate of VEGF by cells, δ is a piecewise function that introduces stochasticity into the model, D is the diffusion constant, and ε is the decay of VEGF. Equation 5 was coupled to a CPM description of endothelial cell migration, and predictions of the model were validated by comparing the patterns of networks simulated to in vitro data of vasculogenesis. Underlying parameter values were varied to explore their impact on endothelial network formation, and thus inform whether hypothesized mechanisms governing network formation were consistent with experimental observations (Vokes et al. 2004).

Guidolin et al. (2009) expanded the work of Merks et al. (2006) by developing a more robust validation procedure. They acquired data on endothelial cell number, length of network branches, and the area occupied by cells for several time points through dedicated experiments. The majority of the model parameters were informed from these experiments; the remainder, such as the number of cell mitoses, was estimated to ensure the morphology of the capillary networks matched the data. With a validated model they were able to qualitatively demonstrate the importance of endothelial cellular proliferation in the process of vasculogenesis. This demonstrates the value of combining computational models with dedicated experimental data to generate robust predictive models.

All the examples considered thus far consider a single cell type in isolation. For realistic in vivo scenarios relevant to peripheral nerve regeneration, multicellular processes are commonplace. Prokopiou et al. (2016) have extended the basic CPM framework to account for different cell populations (different endothelial phenotypes and astrocytes) involved in angiogenesis, to understand how changes in VEGF gradients can impact capillary networks. This is done by considering different values of τ and assigning different values to each cell type such as interaction J energies between the different types of cells in Eq. (3). Furthermore, Prokopiou et al. (2016) considered the role of astrocytes as producers of VEGF which, when considered as chemotactic cues, impact the development of vasculogenic networks. The complex model involves well-characterized mechanisms for which the majority of parameters can be obtained from in vivo and in vitro experiments. However, there are parameters in the CPM that are difficult to measure, especially when considering interactions between cells and the environment, including single cell and cell-cell adhesion parameters (Guidolin et al. 2009). For these parameters, the authors used a sensitivity analysis to identify the range of parameters that agreed with in vitro experiments and other in silico model predictions.

These examples demonstrate the potential of CPM algorithms to describe cellular behavior and their dependence on underlying biochemical features of the system. In order to facilitate the implementation of these models, Cickovski et al. (2007) have developed a graphical interface software called CompuCell3D, which enables implementation, visualization, and analyzation of these modeling frameworks, in

both 2D and 3D. However, CPM are highly computationally expensive and can be challenging to calibrate against experimental data (Metzcar et al. 2019) given the challenges of cell-scale measurements.

A less computationally expensive framework than CPM consists of simulating cellular behavior as random walks using either an off-lattice or lattice-dependent regime. Conversely to the CPM, where at each time it is not required to iterate over all lattice points, for a random walk model, decisions are made at the cellular tips only, as the model simulates each cell individually. Formally, these models can be described by adapting the Langevin equation of the Brownian motion of particles. As stated by Stefanoni et al. (2011), although the movement of cells is radically different from the thermally induced movement of particles suspended in a fluid, models that describe cells using this Brownian motion approach generate realistic descriptions of cell trajectories, in the absence of external guidance. The Langevin equation states that, letting $x(t)$ denoted the position of the cell on the substratum at time t , the movement of the cell tip can be described as:

$$m \frac{d^2x}{dt^2} = -\zeta \frac{dx}{dt} + \sum F(t), \quad (6)$$

where m is the mass of the cell and $\frac{dx}{dt}$ and $\frac{d^2x}{dt^2}$ are the speed and the acceleration of the cell tip. Equation (6) can be interpreted as Newton's second law of motion under the assumption that the cell experiences only two forces: a drag force $-\zeta \frac{dx}{dt}$ with drag coefficient ζ that represents all the forces that impede the movement of the cell and, as in Eq. (6), $F(t)$ accounts for all the stochastic effects acting on the cell. Equation (6) can be rewritten in incremental form (Doob 1942) by writing it in terms of the cell velocity $v(t)$ as:

$$dv(t) = \frac{\zeta}{m} v(t) dt + dB(t). \quad (7)$$

The term $dB(t)$, following the work of Coffey and Kalmykov (2012), is assumed to be a Gaussian distributed stochastic process with average zero and variance αdt , where α is a constant and dt is the time step of each iteration. The position of the cell tip $x(t)$ can be calculated by integrating $v(t)$ with respect to time t and knowing the initial position of the cell (x_0) at time $t = 0$ following:

$$x(t) = x_0 + \int_0^t v(t') dt'. \quad (8)$$

The framework presented contains the main elements required to simulate cellular random motility such as stochasticity and persistence or inertia, and can be extended by adding a deterministic vectorial term to the right of Eq. (5) to capture the role of haptotaxis (Dickinson and Tranquillo 1993; Smith et al. 2004), chemotaxis (Dickinson and Tranquillo 1993; Stokes and Lauffenburger 1991; Tranquillo and Lauffenburger 1987), or galvanotaxis (Schienbein and Gruler 1993).

Random walks have yet to be employed to simulate the multicellular mechanisms behind nerve repair. However, as in the case for CPM, they have been used to study the behavior of central nervous system neurites and endothelial cells in the presence of a variety of cues. In terms of neurites there are three established packages that simulate cellular growth and branching in the central nervous system (Bower and Beeman 2012; Hines and Carnevale 1997; Zubler and Douglas 2009). However, only the Zubler and Douglas (2009) work considers the role of chemical cues on the growth of neurites. The model appropriates Eq. (5) and simplifies further neglecting the inertial term, given that growth speeds are so slow, to give

$$\zeta \frac{dx}{dt} = \sum F(t). \quad (9)$$

The major consequence of Eq. (8) is that the major obstacle to movement is no longer inertia but the friction associated with movement. Now the displacement of cells (Δx) at each time step (Δt) can be approximated via:

$$\Delta x = \left(\frac{1}{\zeta} \sum F(t) \right) \Delta t. \quad (10)$$

The cells grow within a lattice based on the balance of three forces: 1. interaction between the cells, other cells, and any chemical or physical cues present in the environment; 2. the internal cell tension simulated as a spring model that is influenced by metabolic growth, and 3. additional forces that may be relevant to a specific experimental or biological scenario. Similarly to the CPM framework, the role of chemotactic (and other continuum) cues can be incorporated by coupling with the relevant continuum models (for example, Zubler and Douglas (2009)).

Additionally, an important mechanism for peripheral neurite growth is branching, resulting in neuronal morphologies resembling plant roots, which has been captured realistically by several mathematical models. Statistical models (Van Pelt et al. 1997), Markov models (Samsonovich and Ascoli 2005) and Monte Carlo models (Coelho and da Fontoura Costa 1997) can reproduce morphologically accurate branching of neurites; however, these models do not take into consideration the impact of cues on branching rates and have not yet been applied to repair scenarios.

To the best of our knowledge, the only model that seeks to validate a 3D model of neurite growth was developed by Razetti et al. (2018). The model consists of an off-lattice framework with angles of elongation informed stochastically based on the underlying chemotactic environment. In order to mimic experimental observations, a neuronal search mechanism is included by which neurites extend and retract to search for the best direction to take. There are limited data available on the geometries of elongating, repairing neurites after a peripheral nerve injury so in vivo data from *Drosophila* are often used (e.g., Wu and Luo 2006). Using image analysis methods described in Razetti et al. (2016), neuron morphological parameters such as distribution of neuron angles and lengths, number, length, and order of branches were

extracted, and used to inform parameters in the Razetti et al. (2018) model. The robustness of the parameter values was tested by performing the impact of small parameter changes on model predictions, and the model behaviors were consistent with experimental findings. Finally, Razetti and collaborators quantified the impact of neurite branching on neurite growth, demonstrating that when a discrete model, whose parameters might not be derived from physics, is paired with experimental work, the model acquires great predictive potential.

Similar approaches have also been found to be useful in understanding the development of capillary networks, in particular in the case of tumor-induced angiogenesis (for example, McDougall et al. 2002). Although there are fundamental differences in tumor-induced angiogenesis compared to that during PNR, there is commonality in the underpinning biomechanics, biochemistry, and growth frameworks (for example, the role of VEGF as a chemotactic cue for growing endothelial cell tips). As one widely adopted and comprehensive example, McDougall and colleagues (Aubert et al. 2011 and McDougall et al. 2006) have developed a framework called dynamic adaptive tumor-induced angiogenesis (DATIA), where the growth and branching of endothelial tip cells are simulated, following similar hybrid continuum-discrete frameworks as described previously for neurites. The movement of endothelial cell tips is described using a discrete random walk, biased toward areas of increasing/decreasing gradients of chemical and haptotactic cues (described using continuum models, and capturing production and degradation, dependent on the underlying cell populations).

Ultimately, elongating endothelial cell columns form junctions and then networks through which blood flows, oxygenating its surroundings and altering the local environment conditions. All these processes require further adaptations to the discrete-continuum modeling frameworks. For example, the discrete model is updated so that when different endothelial cell tips encounter each other they form links and junctions resulting in “hollow” capillary networks (Anderson and Chaplain 1998). These networks may then sustain blood flow, described using Poiseuille’s law, which the nodal pressures in a network (p_k) to the segment fluxes, Q_k , via

$$Q_j = \sum_{k \in N} M_{jk} p_k, j \in S, \quad (11)$$

where N and S are the set of all nodes and vessel segments in the network, respectively, and

$$M_{jk} = \begin{cases} +\frac{\pi d_j^4}{128 \mu_j l_j}, & \text{if } k \text{ is the start node of the segment } j, \\ -\frac{\pi d_j^4}{128 \mu_j l_j}, & \text{if } k \text{ is the end node of the segment } j, \\ 0, & \text{otherwise} \end{cases} \quad (12)$$

where d_j and l_j are the diameter and the length of the segment j , respectively, and μ_j is the dynamic viscosity of the fluid at segment j .

As a consequence of the conservation of mass, the sum of the flow at each interior node is zero. This can be combined with the network boundary conditions giving the following condition for flow conservation,

$$\sum_{j \in S} L_{ij} Q_j + Q_{0i} = 0 \text{ for } i \in N, \quad (13)$$

where

$$L_{ij} = \begin{cases} -1, & \text{if } i \text{ is the start node of the segment } j, \\ +1, & \text{if } i \text{ is the end node of the segment } j, \\ 0, & \text{otherwise} \end{cases} \quad (14)$$

For all interior nodes, $Q_{0i} = 0$, however, if i is a boundary node, Q_{0i} is the inflow (or outflow if negative). Combining Eq. (11) with (13) yields

$$\sum_{k \in N} K_{ik} p_k = -Q_{0i} \text{ for } i \in N, \quad (15)$$

where

$$K_{ik} = \sum_{j \in S} L_{ij} M_{jk}, \quad (16)$$

providing a linear system to solve for the nodal pressures (Butt 2009). These frameworks may also take into account the non-Newtonian nature of blood at the scale of the microcirculation, for example through the viscosity relationships.

McDougall et al. (2012) have extended the framework described above for endothelial cells to incorporate interaction with glial cells in the context of retinal vascularization. Continuum models for the diffusion, production, and degradation of cellular-specific chemical cues (such as VEGF) were added, including interactions between cell types. Blood perfuses the capillary network and supplies oxygen to the surrounding tissue, promoting proliferation of both cell types. A two-compartment model is developed to describe oxygen dynamics incorporating the average oxygen concentration within each vessel (S_v), and that in the tissue (S_T), through

$$\frac{\partial S_T}{\partial t} = D_{S_T} \nabla^2 S_T + \frac{\pi K}{2L^2} \sum_{\substack{\text{vessel} \\ \text{sources}}} [R_i (S_{V_i} - S_T)] - \sigma_{S_T} S_T, \quad (17)$$

and

$$\frac{\partial S_V}{\partial t} = \frac{F_{Q_E}}{\pi R^2 L} D_{S_T} \sum_{\substack{\text{vessel} \\ \text{sources}}} [Q_j S_{V_j}] - \frac{Q S_V}{\pi R^2 L} - \frac{K}{2R} \sum_{\substack{\text{vessel} \\ \text{sinks}}} [S_{V_i} - S_T]. \quad (18)$$

Equation (17) encompasses diffusion of oxygen in the tissue (parameter D_{ST}), osmotic exchange between the two compartments (with permeability K dependent on the radius R of each vessel i), oxygen removal rate (σ_{ST}) from the tissue.

The work of McDougall and colleagues demonstrates the value of a hybrid discrete-continuum modeling approach in capturing complex biological processes. However, this also presents a challenge in accurately defining the number of parameters based on real-world data. McDougall et al. (2012) make valiant efforts, including comparison against literature-reported values, parameterization, and validation against dedicated experimental data (for example, on retinal architectures at different stages of development). The resultant models have predictive capabilities and, for example, have been used to investigate the effects of transgenic over-expression of VEGF-A on the development of retinal vasculature.

In conclusion, discrete and hybrid discrete-continuum models have powerful potential to describe complex biological phenomena, if developed alongside dedicated experimental studies. Table 1 provides a summary of the models (discrete, continuous, hybrid) discussed here, their comparison against experimental data, and outcomes. Although not developed to describe PNI repair, these frameworks have the potential to incorporate the relevant components outlined in Sect. 1: (1) scaffold/substrate, (2) growth factors, (3) extracellular matrix and (4) therapeutic cells. However, the complexity of hybrid models can reach unmanageable levels and the trade-off between the complexity of the models and their predictive potential requires careful attention. In the next section, we compare discrete and continuum modeling approaches, and propose an approach to address this trade-off.

2.3 Comparing Discrete and Continuum Frameworks

As discussed, and summarized in Table 1, discrete, continuum, and discrete-continuum frameworks have been developed extensively in the literature for a range of disease and repair scenarios, and have the potential to be further developed and applied to describe the dominant mechanisms underpinning nerve regeneration. As in other fields, one might erroneously assume that the more realistic model is the one that is able to incorporate the most complexity (both in terms of numbers of mechanisms, and how they are described). However, increased complexity increases the number of parameters and relationships that must be informed by reliable data in order for the models to have predictive capability. John von Neumann stated that “With four parameters I can fit an elephant, and with five I can make him wiggle his trunk” (Dyson 2004). This quote illustrates that although a more complex model might better fit an underlying dataset, its predictive power might be questionable. When confronted with a complex biological scenario, spanning multiple time and length scales, what is the best strategy to develop an *in silico* model capable of producing reliable predictions? We propose starting by addressing two questions:

Table 1 Summary of the example modeling frameworks of relevance to peripheral nerve injury repair

Authors	Type	Elements simulated	Validation data	Conclusions
Podhajsky and Myers (1995)	Continuum	Interaction between erythrocytes, axons, Schwann cells, and fibroblasts during nerve regeneration	Model simulations match in vitro experimental observations qualitatively	Using a continuum model, it is possible to capture the interaction of different cell populations during peripheral nerve regeneration
Rutkowski and Heath (2002)	Continuum	Influence of porosity of a polymer NRC, pre-seeded with Schwann cells, on nerve regeneration	In vitro experiments to quantify rates of glucose and oxygen consumption by Schwann cells; neurite growth in presence of different NGF concentrations	The optimal porosity of the NRC designed was identified
Prokopiou et al. (2016)	Hybrid: Cellular Potts (discrete) plus reaction-diffusion (continuum)	Angiogenesis in the presence of VEGF type A produced by glial cells	In vivo network morphological data of retinal angiogenesis in neonatal mice	Observed, in vivo endothelial sprout morphologies were reproduced in silico, pointing to the potential dependence on VEGF type A gradients
Guidolin et al. (2009)	Hybrid: Cellular Potts (discrete) plus reaction-diffusion (continuum)	Role of chemotactic cues in endothelial cell tip elongation and vascular network formation	In vitro data from human umbilical vein endothelial cell (HUVEC) cultures in presence of chemotactic factors	Representative physiological behaviors were captured using an in silico model for a variety of conditions
Zubler and Douglas (2009)	Hybrid: 3D random walk (discrete) plus reaction-diffusion (continuum)	Developmental neuronal network formation	Parameters taken from the literature	Based on parameters taken from the literature, the model could generate physiologically reasonable developmental neuronal networks

(continued)

Table 1 (continued)

Authors	Type	Elements simulated	Validation data	Conclusions
Van Pelt et al. (1997)	Discrete: random walk	Neurite morphology	Data taken from the literature on rat cerebellar Purkinje cells	Branching probabilities and morphologies were estimated for postnatal cerebellar Purkinje cells
Razetti et al. (2018)	Hybrid: 3D random walk (discrete) plus reaction-diffusion (continuous)	Uncover how neurites interact to optimize growth	3D morphological in vivo data from <i>Drosophila</i> model	Framework that allows the study of the rules that dictate the growth of neuron populations
McDougall et al. (2012)	Hybrid: 3D random walk (discrete) plus reaction-diffusion (continuous)	Angiogenesis of retinal vasculature in neonatal mice in response to astrocyte migration	In vitro data into nerve and astrocyte migration	The model is able to capture healthy behavior of retinal vascular formation.

1. What is the length scale required for the model?
2. What data is available to inform the model?

The first can illuminate which framework to use, balancing an easier to analyze but more phenomenological, continuum model, or a smaller-scale, discrete model. In the context of nerve engineering, the choice of these two frameworks centers on understanding if the research question requires information on the spatial interactions at the scale of individual cells. Continuum models have been shown to realistically capture bulk cell population properties, such as cell population growth and interaction with chemotactic cues (Rutkowski and Heath 2002). However, interactions at the cell-scale are also important in understanding and describing regeneration. Therefore, a choice needs to be made to ensure the model framework is tailored to the research question at hand.

Within discrete modeling frameworks, we have presented and discussed two approaches. The Cellular Potts Model (CPM) is more of a “black box” approach, compared to the more flexible random walk, which can explicitly incorporate specific biomechanical and biochemical mechanisms. There is also an associated balance between the computational complexity and expense of the approaches. The CPM requires several cell updates per iteration, therefore, for simulations that have a large number of cells, computational expense may become intractable. Alternatively, the random walk is less computationally expensive, but includes a larger number of parameters that must be informed by experimental data.

The choice of the model framework can also be informed by the data available, as all these frameworks require different forms of data for validation. A continuum

framework requires information on diffusion rates for each species (cells, chemical species), cell proliferation and death rates, release of chemical factors, and their uptake and/or degradation, among other features. By comparison, a CPM employs a “black box” approach, disconnected from the underpinning physics of the system. This means that common parameters to all CPMs, e.g., the resistance of cells to compression (λ_A), can be fitted to ensure the model outputs match the data. The random walk more closely represents and incorporates known understanding of the physics underpinning the system, therefore requires more tailored data than the CPM. When simulating the growth of cells such as neurites and capillaries in 3D, a full quantitative analysis of cellular movement is required to inform the model, including information on cell numbers, directions, and branching patterns. Due to its flexibility, the random walk model can be used to reproduce data at the cellular level as well as ensuring the model outputs match the data available. However, the flexibility of the random walk comes with the cost of an increase in the number of parameters, which in extremum becomes unmanageable.

In particular cases, a hybrid approach is a good compromise, for example to investigate the impact of the output of a continuum model on discrete objects (for example, discrete models of individual cells or cell tips, which are biased toward higher concentrations of diffusible chemotactic species). Although these models might better approximate the complexity of a model, their data requirements are also harder to attain.

Furthermore, research suggests that the predictions of continuum and agent-based models of cell populations disagree in certain parameter spaces. Krause et al. (2017) demonstrated that in some cases continuum and lattice-based models of porous engineered tissue can result in different qualitative outcomes. In particular, the partial differential equation model exhibited less spatial heterogeneity of cell density than the lattice model, and this effect was exaggerated in certain parameter regimes. This serves as one example where the scale of length scale of the model will determine its predictive potential.

In summary, through a well-reasoned process that addresses the balance of length scale of the research question, and data availability, it is possible to arrive at a well-suited model. A summary of the process underlined in this section is presented in Fig. 6.

2.4 Parameter Optimization and Sensitivity Analyses

As any model becomes more complex, it becomes harder to define an increasing number of parameters and next we describe methodologies and considerations for defining model parameters, and evaluating their impact on model predictions.

In the absence of concrete data, many models exhibit “sloppy” behavior (Brown and Sethna 2003), described as: i) a large number of poorly determined or completely unknown parameters; ii) the models have simplified dynamics, i.e., they do account for all the mechanisms involved in a system and iii) the interactions between parameters might not be fully described. These characteristics are

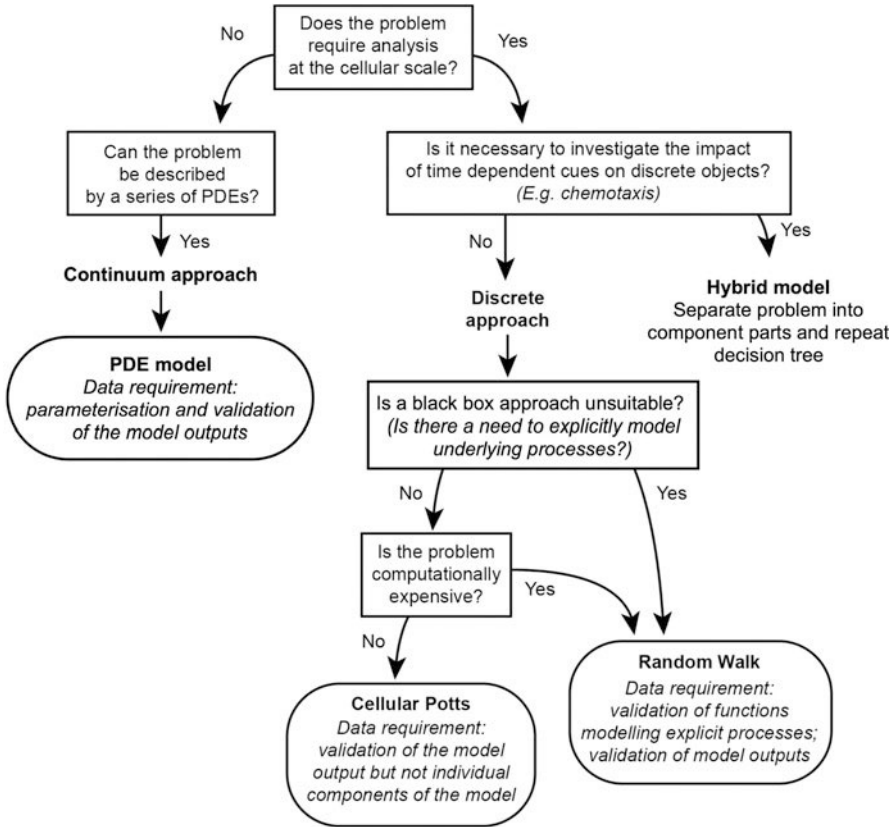


Fig. 6 A decision framework to pair the adequate mathematical modeling strategy to the aim of the research question and the data available

commonplace in the systems biology literature. Gutenkunst et al. (2007) explored 17 system biology models as diverse as circadian, metabolism, and signaling systems and examined the sensitivity of each model to underlying parameter changes. All these models appeared to demonstrate “sloppy” parameter sensitivity. The authors found parameter measurement to be efficient, it must be both very precise and complete. Gutenkunst et al. (2007) concluded that researchers should focus not on the quality of the fits associated to individual parameter values, but perform collective parameter fits to different sets of data and focus on quantifying the quality of predictions of the model through sensitivity analysis (SA) algorithms.

Another challenge of parameterizing mathematical models of biological processes is the large range of possible parameter values, and associated challenge of refining that range in a computationally efficient, yet reliable manner. Particle Swarm Optimization (PSO) was devised by Eberhart and Kennedy (1995) and uses an algorithm to explore a parameter space, inspired by the properties of a flock of

birds looking for food. The algorithm starts by placing particles in the search space (this represents initial estimates for all parameter values), and evaluating a function that represents the feature to be optimized (e.g., the error between model predictions and experimental data). Each particle evaluates a potential move by comparing the value of the function at the previous positions to the best (closest to the optimal value) location found by one or more neighbors, which is perturbed by a stochastic term. Progressively, particles “swarm” to the optimum values desired, e.g., the parameter values for which the model better matches the data.

The particles are placed in a D -dimensional space, where D is the number of parameters being optimized. Three vectors are defined: the current position $\vec{x}_i$, the previous best position $\vec{p}_i$, and the velocity $\vec{v}_i$. As described by Poli et al. (2007), every iteration of the algorithm starts with evaluating the model with the parameters at the current position of the particles. If a best position is found compared to the one that was previously found, then the coordinates and value at those coordinates are stored in $\vec{p}_i$ and p_v , respectively. This is followed by moving the particles by adding the vector $\vec{v}_i$ to the current positions $\vec{x}_i$. Therefore, the aim of the algorithm is to adapt $\vec{v}_i$ so that the particles move in the direction of $\vec{p}_i$ in the hopes of finding progressively better p_v . The algorithm is computed until a user-defined stopping criterion is met. Vaz and Vicente (2007) provide the most widely adopted implementation of PSO, via a software package usable in a variety of platforms and languages to facilitate its implementation.

After an accepted parameter set is found via optimization, it is important to test the impact of these parameter values on the predictability of the model. This can be done through sensitivity analyses (SA), which, as stated Saltelli et al. (2002), apportion the uncertainty of the output of a model to different sources of uncertainty of the model parameters, as well as quantifying the dependency of the model on the data available. Iooss and Lemaître (2015) have described the aims of SA as determining the most and least influential input variables to the behavior of the output. In addition, SA can quantify the interactions between model parameters and how they impact the predictions of a model. Commonly, SA is performed locally within a small range of parameter values and each parameter is changed individually, computed via a system of derivatives (S) that quantifies the impact of an input (X) on the output of the model (Y) as $S_i = Y/X_i$. Rabitz (1989) uses this process to determine kinetic constants in the context of a chemical system, for example. Such processes are commonly found across the literature as a sensitivity analysis method, however, they have been found by Saltelli et al. (1999) to be inappropriate to quantify the impact of parameter uncertainty on outputs.

A more suitable strategy consists in exploring an entire range of parameter values, by sampling the parameter space through linear combinations of parameter values and testing their impact on the model predictions. These methods are termed global, to contrast with the local methods, where only one parameter value is changed at a time, and are reviewed by Saltelli et al. (2002). The most commonly used approach is called the Morris method, which establishes a hierarchy of parameter uncertainty effects on the model outputs and, therefore, is called a screening method. The method, as implemented by Saltelli et al. (2002), consists

in varying each parameter (p_i where $1 \leq i \leq k$) of the model output function $f(p_i)$ individually within a defined parameter space. The function $f(p_i)$ is defined to evaluate how model output $M(p_i)$ affected by varying parameters. The (p_i) can account for a number any outputs of interest (T_o), such as, the number of neurites that transverse an NRC, the number of braches per neurite, and the number of intersections between endothelial cells. Often a root mean square (RMS) error was used, between the outputs for the original parameter values, p_o , and the outputs for the new parameter values, p_i , as:

$$f(p) = \sqrt{\sum \frac{[M(p_o) - M(p_i)]^2}{T_o}}. \quad (19)$$

For each p_i , a maximum (p_{max}) and minimum (p_{min}) are chosen. The parameter space is explored by establishing a grid where each parameter can take n uniformly distributed values between the extremities of the space, defined as

$$p_{i,m} = p_{i,min} + m \frac{p_{i,max} - p_{i,min}}{n - 1}, \quad (20)$$

where m is an integer between 0 and $n - 1$. A k -dimensional grid with n levels is formed with $p_{i,m}$ values as entries, let $\vec{p}$ be the current point on the grid corresponding to the complete set of parameters for which the model is tested and Δ_i be defined as

$$\Delta_i = m_{\Delta,i} \frac{p_{i,max} - p_{i,min}}{n - 1}. \quad (21)$$

Then a change of the current point on the grid to a new one can be defined as

$$\vec{p} = [p_1 + \Delta_1, \dots, p_i + \Delta_i, \dots, p_k + \Delta_k] \quad (22)$$

with values $m_{\Delta,i}$ defining the number of grid point steps by which the hump occurs for each parameter. In the Morris method only one parameter is varied in turn by Δ_i . For each change the gradient $d_i(\vec{p})$ is calculated as:

$$d_i(\vec{p}) = \frac{f(p_1, \dots, p_i + \Delta_i, \dots, p_k) - f(\vec{p})}{\Delta_i}. \quad (23)$$

Each $d_i(\vec{p})$ is called an elementary effect (EE), with a total of $n^{(k-1)}(n-1)$ EE's.

Starting at a randomly chosen base point, $\vec{p}^0$, a trajectory of $k+1$ values of $\vec{p}$ are generated by incrementing each parameter p_i by $\pm \Delta_i$ in turn, so that each point differs from the precious value in only one parameter. From two points, and EE is calculated that pertains to one parameter p_i , and the process is repeated for r trajectories, requiring a total of $r(k+1)$ function evaluations.

From the set of all EEs, the standard deviation (σ) and the mean (μ) are calculated for each p_i . A large μ establishes that the parameter has a large effect on the model output, while a small μ indicates a small effect. Additionally, the σ can be used to identify those parameters, which have an independent effect (small σ) and those that interact with other parameters (large σ). An off-the-shelf implementation of the Morris method for different coding languages has been developed by Tarantola and Becker (2016) titled the SIMLAB package.

This well-established method, like many others in the literature, utilizes the variance of the output in response to the variance in parameter values as the measure of impact. There are several reasons for ready uptake of these methods, as compiled by Pianosi and Wagener (2015), including: they are independent of the relationship between inputs and outputs, they can be used for ranking the importance of parameter values, and they are easy to implement and interpret. However, as commented by Saltelli et al. (2010), in order for variance-based methods to be accurate, a large number of simulations of the model are required (e.g., as demonstrated by the work by Baroni and Tarantola (2014), a model with $k = 5$ would require thousands of model evaluations). Additionally, the assumption that the variance of the parameters correlates with the uncertainty of the outputs might not be entirely correct (for example, Borgonovo (2011) demonstrated this not to be true, when the output distribution is multi-model or highly skewed).

These limitations have led to alternative measures to make sensitivity analysis “moment independent,” i.e., measures that do not use moment-specific indices of the output distribution and therefore are independent of its shape. These methods make use of the probability density function (PDF) of the output distribution, rather than its variance, by quantifying the changes the PDF experiences when the variance of a certain parameter is removed (for example, entropy-based methods (Liu et al. 2006) or δ -sensitive measures (Plischke et al. 2013)). Although PDF-dependent methods are more accurate, their implementation is more complex. This is because for each uncertainty input at least one PDF needs to be estimated. Consequently, when it is necessary to compute multiple conditioning values the number of computations becomes, once again, excessive. Consequently, Pianosi and Wagener (2015) proposed a method called PAWN (derived from the authors names) that seeks to simplify the implementation of a moment-independent strategy by developing a method whose metric is dependent on the cumulative density function (CDF). The algorithm consists in, firstly, evaluating the unconditional CDF ($F_y(y)$) of the output of the model by varying the M parameters ($\mathbf{x}$, $\mathbf{x} = [x_1, x_2, x_3, \dots, x_M]$) within a user defined range at random, then, similarly a conditional CDF ($F_{y|x_i}(y)$) is calculated for each parameter by maintaining one of the parameters constant in turn and varying the remaining randomly. For the latter CDF the process is repeated a number of times for different constant values of each parameter (referred to as the conditioning points). The two CDFs are compared using a Kolmogorov-Smirnov (KS) statistic,

$$KS(x) = \max_y |F_y(y) - F_{y|x_i}(y)|. \quad (24)$$

The parameter to which the model is more sensitive to is the one for which the constrained CDF varied the most from the unconstrained CDF. The metric devised has the advantage, when compared to the algorithms dependent of the PDF, of requiring less iterations and their outputs can be visualized graphically. Furthermore, a toolbox has been provided that facilitates the implementation and dissemination of their method available for many platforms.

However, Puy et al. (2019) have found that the PAWN measure is sensitive to the method's parameters, which include the number of parameter samples to estimate the unconditional CDF (N_C), the number of samples to develop the conditional CDF (N_U), and the number of conditioning points (n_C). Regardless, it is agreed that there are many advantages of this model compared to moment-dependent methods.

Sections 2.2 and 2.3 have discussed the merits of different modeling approaches, and options for parameter setting and evaluation. Although these approaches have rarely been used in the context of PNI repair, they have the potential to be impactful in informing tissue engineering strategies, alongside counterpart experimental studies. To demonstrate this potential, next we present two case studies: the first seeks to understand the role of cell seeding in an NRC through a continuum modeling approach, and the second explores how to position material cues in an NRC to accelerate neurite growth via a discrete approach.

3 Case Studies on the Use of Mathematical Models in PNI Repair

Having presented the state-of-the-art in mathematical modeling relevant to PNI, here we present two case studies, which demonstrate how mathematical modeling may be used to inform design criteria for NRCs. We focus on Engineered Neural Tissue (EngNT), which has been developed at UCL by Prof. James Phillips (UCL Centre for Nerve Engineering). EngNT organizes therapeutic cells and matrix molecules into stable aligned constructs, resulting in precise positioning and orientation of therapeutic cells and matrix components (e.g., Georgiou et al. 2013 and Martens et al. 2014). This provides a robust, versatile and reproducible system in which to test hypotheses around spatial distribution of cellular and material components, and this is highly amenable to integration with a mathematical modeling approach.

We present two case studies. The first uses mathematical modeling as a tool to identify cell seeding criteria in EngNT with a view to optimizing production of pro-angiogenic factors as a pre-cursor to regenerative angiogenesis (and following the approach reported in (Coy et al. 2020)). The second explores how material cues can be used to accelerate neurite regrowth. In both scenarios, we focus on the geometry of a typical preclinical repair scenario – a rat sciatic nerve model, which is the commonly used in vivo model used for PNI repair.

3.1 The Role of Cell-Seeding in Producing Pro-Angiogenic Factors: A Continuum Approach

3.1.1 Introduction

EngNT (and NRCs more generally) may include therapeutic cells, which serve numerous roles, including secreting of pro-angiogenic growth factors that can aid vascularization of the repairing tissue (as well as neuronal regrowth). Although extensive research has focused on the choice of therapeutic cells seeded within an NRC, short-term cell survival, longer-term regenerative angiogenesis, and the role of cell seeding densities and distributions have received less attention. The basic provision of nutrients and removal of potentially toxic metabolites via adequate vasculature is essential for the sustenance of implanted engineered tissue. Without perfusion of blood vessels, tissue must rely on limited oxygen diffusion for survival. This can be sufficient to support tissues that are relatively thin such as engineered skin and corneal tissue, but poses a major barrier to the clinical translation of “3D” engineered tissues with a greater volume: in these cases, hypoxia can lead to the widespread death of cells seeded in the core (Novosel et al. 2011). Thus, the issue of cell survival is intrinsically linked to that of vascularization.

Additionally, experimental evidence suggests that during peripheral nerve repair, endothelial cell migration and blood vessel growth precedes neuronal growth, and that blood vessels provide mechanical and directional support that enable the migration of cords of SCs (Cattin et al. 2015), thereby further underlining the importance of fast and directional vascular regeneration in this context.

Vascular endothelial growth factor (VEGF) is generally regarded as the most important angiogenic factor. As well as promoting vascular permeability and basement membrane degradation during the initial stages of angiogenesis and acting as a mitogen for endothelial cells, it has been demonstrated that the concentration and gradient steepness of VEGF affects the rate and directionality of endothelial cell migration (Barkefors et al. 2008; Odedra et al. 2011; Wu et al. 2014), reiterating the importance of the spatial dimension for this work.

In this case study, it is hypothesized that the use of specific initial densities and distributions of seeded cells could improve functional outcomes by enhancing cell survival, as well as the generation of gradients of VEGF necessary for vascularization, in implanted NRCs. This is backed up by existing studies, which suggest that the use of particular cell densities could enhance axonal regeneration (Guenard et al. 1992; Mosahebi et al. 2001).

In the case of cell survival, it is speculated that there is a potential optimal seeding cell density at which enough cells survive for the tissue to remain useful, while limiting the number of cells that die, thus reducing the waste of excessive cell seeding. Additionally, placement of cells could be optimized to avoid high metabolic needs in central portions of the tissue that are more likely to become hypoxic.

In terms of the production of VEGF, the rate of increase of VEGF in a portion of tissue will largely be determined by the number of VEGF-secreting cells present,

establishing a direct link between cell density and VEGF concentration. Evidence suggests that cellular VEGF expression is at least partly regulated by environmental oxygen concentration, with VEGF production increasing in low oxygen conditions (Lafosse et al. 2016). Other factors such as glucose concentration can also affect the rate of cellular VEGF secretion but were assumed to be non-limiting in this study.

Therefore, to investigate the impact of different cell seeding strategies upon cell survival and VEGF concentrations, a spatiotemporal mathematical model was developed to describe and predict the interactions between cell density, oxygen concentration, and VEGF concentration in the initial period (0–5 days) post-implantation.

3.1.2 Mathematical Model

Given the goal of predicting the spatial distribution of cells and angiogenic factors at the scale of the tissue, we develop a continuum mathematical model that tracks tissue-level distributions in space and time. We propose a mathematical model that describes the interactions between viable cell density n , oxygen concentration c , and VEGF concentration v in hydrogel. We focus on collagen-based gels, although the approach is readily generalized to other matrix components. The governing equation for cell density incorporates a cell proliferation term, dependent upon oxygen and mediated by a function of cell density, and a cell death term dependent only upon cell density:

$$\frac{\partial n}{\partial t} = \beta cn \left(1 - \frac{n}{n_{max}} \right) - \delta n. \quad (25)$$

Here β is the cell proliferation coefficient, n_{max} is the maximum cell density, and δ is the cell death coefficient.

The oxygen concentration governing equation includes a diffusion term and an oxygen uptake term dependent upon the cell density,

$$\frac{\partial c}{\partial t} = D_{c_g} \nabla^2 c - Mn \frac{c}{c + \bar{c}}, \quad (26)$$

where D_{c_g} is the diffusivity of oxygen in collagen gel, M is the oxygen metabolism constant, and $\bar{c}$ is the concentration at which the oxygen consumption is half-maximal.

Finally, the VEGF governing equation incorporates diffusion, secretion by the cells, and decay:

$$\frac{\partial v}{\partial t} = D_{v_g} \nabla^2 v + \alpha n \left(\frac{V_m + 1}{2} - \frac{V_m - 1}{2} \tanh(B_v(c - c_h)) \right) - d_v v, \quad (27)$$

where D_{v_g} is the diffusivity of VEGF in collagen gel, α is the baseline VEGF secretion rate, V_m determines the factor by which the VEGF secretion rate is upregulated at low oxygen concentrations and B_v determines the gradient of

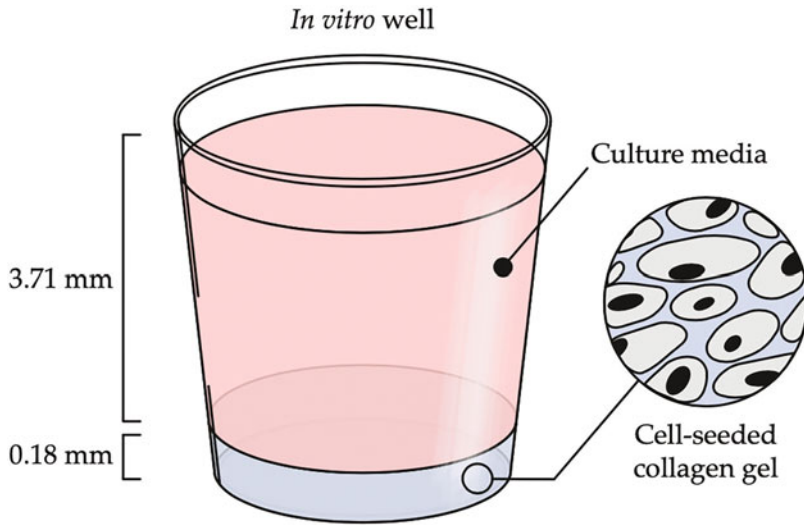


Fig. 7 Cell Culture well schematic. This setup was used to generate in vitro data on the response of therapeutic cells to varied seeding and ambient oxygen conditions

upregulation, c_h is the hypoxia threshold value for VEGF expression upregulation, and d_v is the VEGF decay rate.

To facilitate the development and parameterization of the model, a set of in vitro experiments were specifically designed and carried out, as reported in (Coy 2020). We focus on Schwann cell-like differentiated adipose-derived stem cells (dADSCs) as one option for therapeutic cells (although again the approach is readily extended to other cell types). Collagen gels seeded with dADSCs were cast and stabilized within 96-well plates at 5 densities (n_0), immersed in media, as presented in Fig. 7, and cultured at 5 ambient oxygen concentrations (c_a) for 24 h, then cell viability, VEGF concentration, and cell proliferation were measured. Statistical analysis of the data was used to inform the functional forms used in the governing equations, and the cell viability data and VEGF data were then separated into training and validation data sets for use with the mathematical model.

Parameterization was carried out by first applying the mathematical model to a geometry representative of the wells used in the in vitro experiments within the finite element modeling software COMSOL Multiphysics (COMSOL Multiphysics® v. 3.5. www.comsol.com. COMSOL AB, Stockholm, Sweden). Adaptations were made to the terms of the mathematical model to describe interactions in the media adjacent to the gel: for example, the diffusivities were replaced with corresponding aqueous diffusivities, and the cell density governing equation was not extended to the media portion of the geometry, thus eliminating VEGF secretion and oxygen metabolism in that region.

Parameter values were assigned according to the literature where known; otherwise, they were prioritized for parameterization. Boundary and initial conditions

representing the experimental scenario were applied and the sparse nonlinear optimizer algorithm was used to optimize the simulated values of the mean cell density in the gel and VEGF concentration in the media against the corresponding experimental data in the training data subset, thereby deriving values for the remaining unknown parameters.

3.1.3 Model Parameterization

An optimization strategy was employed to identify the parameter space where model predictions best fit the in vitro data described in Sect. 3.1.2. This process took two phases, the first focused on parameterizing Eqs. 25 and 26 since they are mutually dependent. Then with the found parameters for cell density and oxygen defined Eq. 27 was parameterized. The Sparse Nonlinear Optimizer (SNOPT) function was used to minimize the two different least squares objective function between model prediction for cell density ($\bar{n}_g$) and VEGF ($\bar{v}_m$) and the experimental measurements (n_{exp} and v_{exp} for cell density and VEGF, respectively) for each of the two stages of parameterization, i. e., LS_n and LS_V defined as:

$$LS_n = \sum_{n_{exp} \in S_T} (n_{exp} - \bar{n}_g)^2 \quad (28)$$

$$LS_V = \sum_{v_{exp} \in S_T} (v_{exp} - \bar{v}_m)^2, \quad (29)$$

where S_T is the experimental optimization dataset. In order to avoid an overfit, the data set was divided into parameterization (S_T) and validation (S_V) data, as presented in Fig. 8.

This process demonstrated that, qualitatively, the model reproduces the general trend of the experimental data, showing a good clear increase in viable cell density with ambient oxygen concentration, as the initial cell density increases.

Additionally, it can be observed that there is a local maximum at 3% oxygen concentration for cell densities above 39×10^6 cells/ml for both Fig. 8A and B. This may suggest a link between these phenomena: the relatively large number of cells at 3% oxygen (in comparison to 1% and 5%) at these values of n_0 could account for the correspondingly high VEGF concentration.

3.1.4 Optimization of Seeding Parameters in an In Vivo Repair Scenario

The same model (Eqs. 25 and 26) was translated to a cylindrical geometry (r, z), as seen in Fig. 9, in COMSOL representing a dADSC-seeded collagen NRC, with length L and radius R_2 (equal to the sum of the radi of the collagen NRC [R_1] and the surrounding sheath [T]). The boundary conditions corresponding to the implantation of the NRC in vivo were applied.

Simulations were run using a variety of initial seeded cell densities and distributions, to predict which cell seeding densities and distributions (n_0) may enhance cell survival and the generation of VEGF gradients during the first 5 days

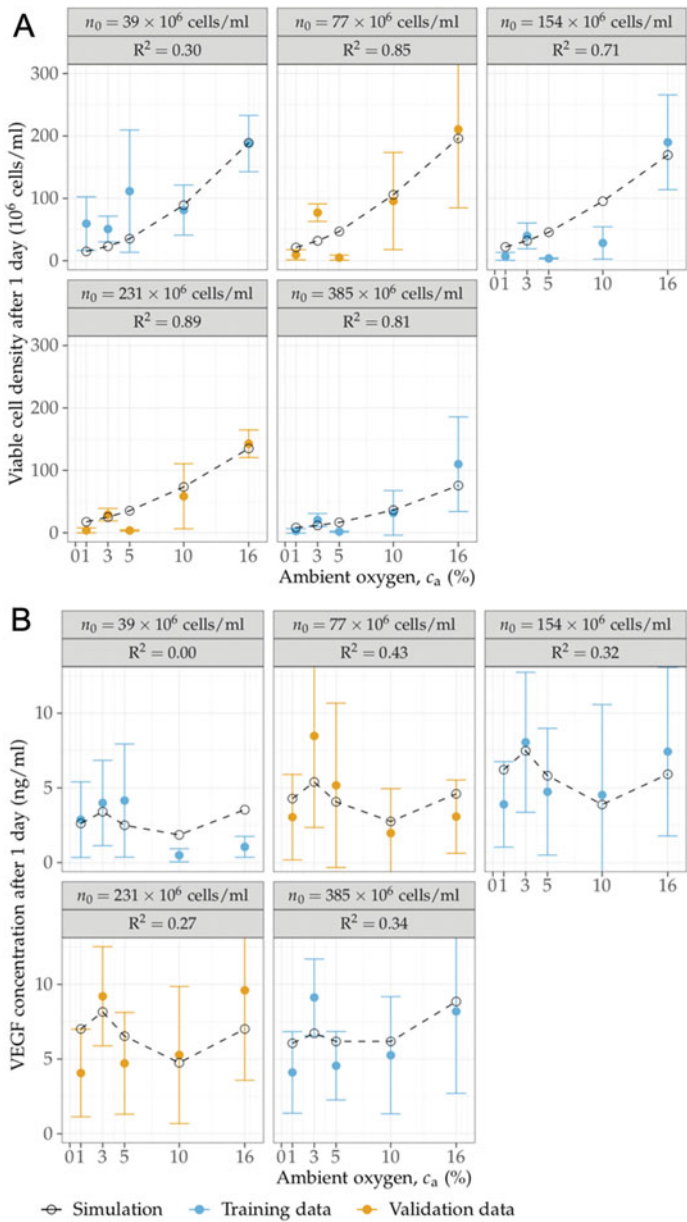


Fig. 8 (a) Simulated values of the mean viable cell density over the gel and (b) the mean VEGF concentration over the media $\bar{v}_m$ after 1 day, produced by the parameterized cell-solute model, approximate the general trends demonstrated by both the training experimental data S_T used during the optimization process (blue), and the validation experimental data set S_V (yellow). Error bars represent standard deviations and R^2 the coefficient of determination of the model predictions compared to in vitro data values

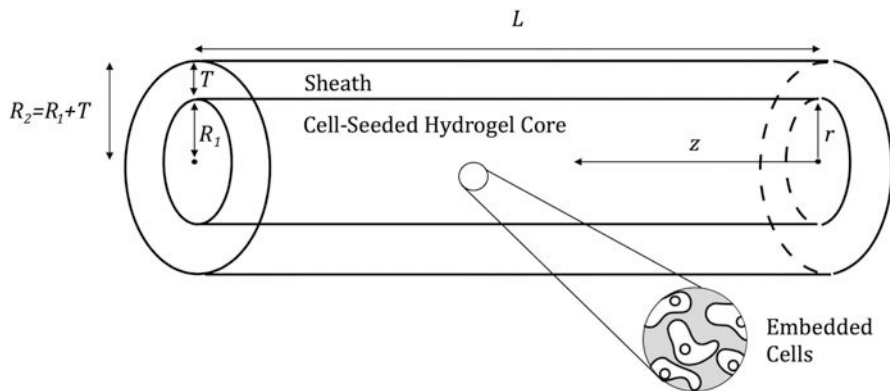


Fig. 9 Schematic of the cylindrical NRC geometry with surrounding sheath, representative of a typical in vivo PNI repair test scenario. The cell-seeded collagen hydrogel forms the core of the NRC, and the evolution of the chemical species (oxygen, VEGF) are predicted throughout this core and surrounding sheath

post-implantation in vivo. The mean ($\bar{n}$) and standard deviation (n_{SD}) across the whole device was used as a measure of cell viability, similarly the same was assumed to quantify the level of VEGF production ($\bar{v}$, v_{SD}) presented, as given in Fig. 10.

Examination of how $\bar{n}$ varies over time reveals that over the initial 12 h NRCs seeded using the highest cell densities maintain the highest values of $\bar{n}$; however, this pattern is somewhat reversed by the 1-day time point. The simulated mean viable cell density $\bar{n}$ across the NRC after 1 day (Fig. 10A) increases monotonically as the seeding cell density n_0 increases from 10×10^6 cells/ml until a maximum is reached at 88×10^6 cells/ml. From there, $\bar{n}$ decreases to a minimum at $n_0 = 400 \times 10^6$ cells/ml.

Regarding VEGF, the simulated mean concentration $\bar{v}$ across the NRC geometry after 1 day achieves a maximum at $\arg \max_{n_0}(\bar{v}) = 236 \times 10^6$ cells/ml (Fig. 10B). The standard deviation of the VEGF concentration v_{SD} achieves a maximum at $\arg \max_{n_0}(v_{SD}) = 267 \times 10^6$ cells/ml. The values of $\arg \max_{n_0}(\bar{v})$ and $\arg \max_{n_0}(v_{SD})$ are consistently similar in value across time, corresponding to the generation of high levels of VEGF in the center of the construct, which increase both v and v_{SD} .

Notably, at the 5-day time point almost no VEGF remains. This corresponds to the fact that the number of viable cells has also dropped dramatically by this time point, resulting in a reduction in the total production rate of VEGF, while VEGF generated at earlier time points has decayed.

Overall, it appears that when a uniform initial cell seeding strategy is used in combination with an impermeable sheath, the value of n_0 required to maximize the amount of VEGF and the steepness of the VEGF gradient after 1 day (236 to 267×10^6 cells/ml) is higher than that required to maximize the viable cell density after 1 day (88×10^6 cells/ml), suggesting that a compromise between the two is required if both high levels of cell survival and steep VEGF gradients are desired. For example, using $n_0 = 170 \times 10^6$ cells/ml generates a relatively steep VEGF gradient

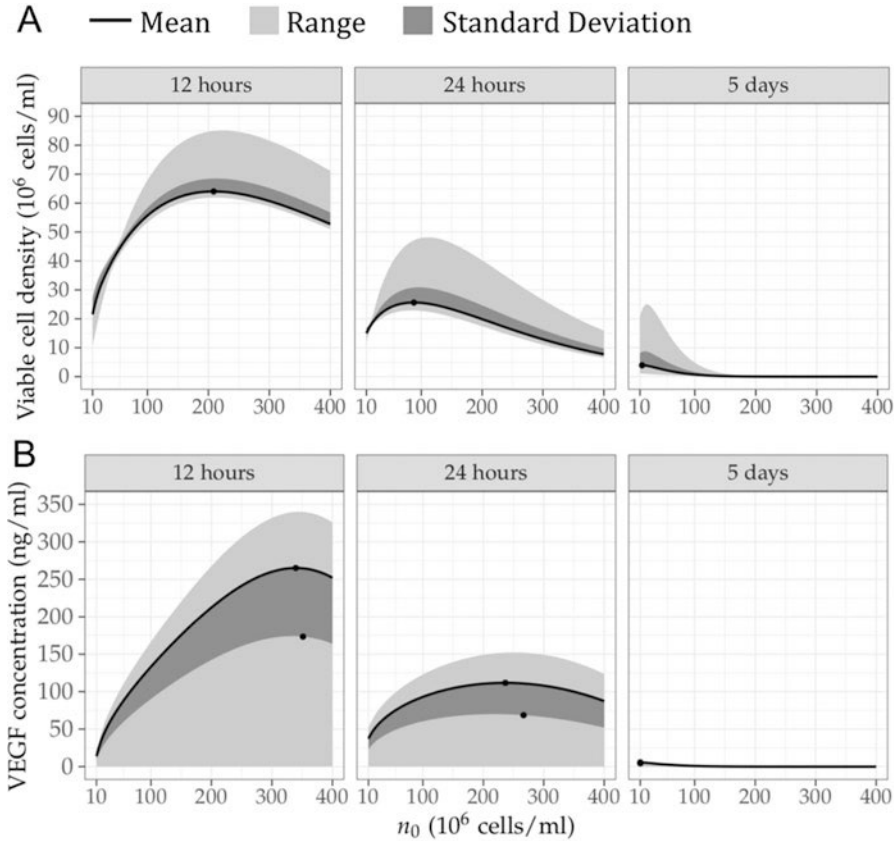


Fig. 10 Simulated viable cell density (A) and VEGF concentration (B) across the NRC geometry for different uniform initial cell densities; black points indicate the positions of the maximum values of mean density $\bar{n}$, mean concentration $\bar{v}$, and maximum VEGF standard deviation v_{SD} . After 12 h, $\arg \max_{n_0}(\bar{n}) = 200 \times 10^6$ cells/ml, $\arg \max_{n_0}(\bar{v}) = 339 \times 10^6$ cells/ml, and $\arg \max_{n_0}(v_{SD}) = 351 \times 10^6$ cells/ml. After 1 day, $\arg \max_{n_0}(\bar{n}) = 88 \times 10^6$ cells/ml, $\arg \max_{n_0}(\bar{v}) = 236 \times 10^6$ cells/ml, and $\arg \max_{n_0}(v_{SD}) = 267 \times 10^6$ cells/ml. After 5 days, $\arg \max_{n_0}(\bar{n}) = 13 \times 10^6$ and $\arg \max_{n_0}(\bar{v}) = \arg \max_{n_0}(v_{SD}) = 10 \times 10^6$ cells/ml (the lowest value simulated)

while sustaining a higher density of viable cell after 1 day than initial densities of greater than 200×10^6 cells/ml. However, it is not certain that maximizing the average concentration of VEGF and the steepness of the VEGF gradient is necessarily the correct strategy to adopt to encourage vascularization and thereby aid neuronal repair.

3.1.5 Sensitivity Analysis to Inform NRC Design Parameters

Sensitivity analysis was performed at two levels. Firstly, local sensitivity analysis was conducted for the cell-solute model using a “one factor at a time” (OAT) technique, in which one parameter is varied at a time while all others are fixed,

Table 2 Parameters varied for local sensitivity analysis

Cell density equation parameters	
Proliferation rate	$\beta = 2.2. \times 10^{-4} \text{ m}^3/\text{mol/s}$
Cell death rate	$\delta(n_0) = \delta_0 + \delta_1 n_0 \text{ 1/s}$ $\delta_1 = 9.1256 \times 10^{-14} \text{ ml/cell/s}$
Maximal cell density ²	$n_{max} = 4 \times 10^8 \text{ cells/ml}$
VEGF concentration equation parameters	
VEGF degradation rate	$d_v = 29.874 \times 10^{-6} \text{ 1/s}$
Hypoxia threshold for VEGF secretion	$c_h = 6.281 \times 10^{-8} \text{ mol/ml(4.77\%)}$

and thus the individual effects of the parameters upon the model output are measured. Each parameter in the governing equations was varied by up to 50% of their final values and the impact was quantified on the total viable cell density fit R^2 , defined as the sum of the R^2 values calculated for each of the initial densities n_0 , the total VEGF concentration fit R^2 , and the sum of these two values, the total model fit R^2 . The parameters that had larger impact are compiled in Table 2, and their influence on the simulations from Fig. 10 can be observed in Fig. 11.

Varying the proliferation rate parameter β was found to result in the largest change in the total viable cell density fit R^2 , and Fig. 11A shows how increasing β by 50% does induce a more drastic change in the model fit to the VEGF concentration data than changes on a similar scale applied to some other parameters.

Secondly, the 3D model predictions were tested by varying boundary conditions within a physiologically realistic range, i.e., the initial oxygen (c_0 , [1, 21] %) and VEGF (v_0 , [0, 10000] pg/ml) concentrations across the NRC geometry and the initial concentrations in the surrounding tissue for both oxygen (c_{tissue} [1, 10] %) and VEGF (v_{tissue} [0, 1000] pg/ml), to investigate the impact upon the model predictions (Fig. 11).

Increasing c_0 across this range decreased the value of $\arg \max_{n_0}(\bar{n})$ after 1 day from $120 \times 10^6 \text{ cells/ml}$ at 1% to $87.9 \times 10^6 \text{ cells/ml}$ at 21%. Both $\arg \max_{n_0}(\bar{v})$ and $\arg \max_{n_0}(v_{SD})$ also decreased at a similar rate across this range of c_0 . However, the corresponding maximal values of $\bar{n}$, $\bar{v}$ and v_{SD} changed by only a relatively small amount. These results imply that cell can be uniformly seeded at a lower density within constructs with higher initial oxygen concentrations to achieve similar values of $\bar{n}$, $\bar{v}$ and v_{SD} after 1 day as constructs with a lower initial oxygen concentration seeded with a greater density of cells. This suggests that one way of minimizing the number of cells required could be to incubate tissue engineered constructs within high oxygen environments prior to implantation. This hypothesis could be investigated experimentally in the future.

On the other hand, varying the initial VEGF concentration v_0 within the NRC over a range of 0 to 1000 pg/ml had no effect on any of the previously mentioned metrics at the 1-day time point. This is because any VEGF in the NRC at the outset decays almost entirely over 24h: the VEGF degradation rate is set at $d_v = 29.874 \times 10^{-6} \text{ s}^{-1}$, equivalent to a half-life of approximately 6.5 h. Increasing v_0 across the designated range not impact the simulated VEGF concentration values after 24 h, motivating the use of $v_0 = 0$ across the simulations. However, the initial

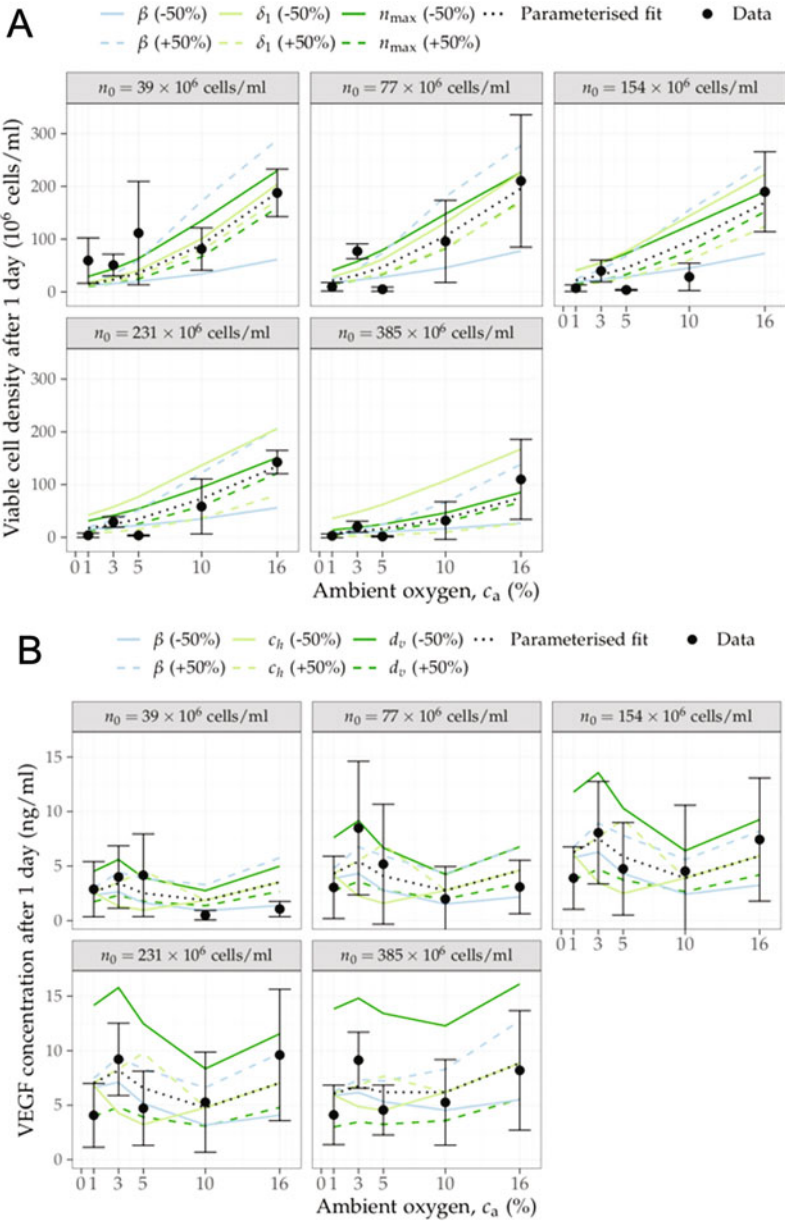


Fig. 11 Black dashed line indicates the final, parameterized model fit; error bars indicate the standard deviations of the viable cell density data. (A) The impact of increasing and decreasing β , δ_1 , and n_{max} by 50% of the values found during optimization, on the model fit to the viable cell density data. (B). The impact of increasing and decreasing β , c_h , and d_v by 50% of the values found during optimization, on the model fit to the viable cell density data. Black dashed line indicates the final, parameterized model fit; error bars indicate the standard deviations of the VEGF concentration data

VEGF concentration, v_0 , does have the potential to alter the concentration of VEGF in the construct over the first few hours after implantation. In this case, the magnitude of v_0 is likely to be smaller than the concentrations generated by the implanted cells, and therefore may not have a great effect on signaling. The latter conclusion relies on the form of the VEGF reaction term presented in Eq. 27, developed based on trends observed in the experimental measurements. Future computational studies could investigate the use of time-released VEGF delivered via capsules using a similar approach, in which case the concentration of delivered VEGF would likely be much higher than the 0–1000 pg/ml range simulated here, and therefore have a greater impact upon the results and may require adapting the functional forms of the reaction terms in Eq. 27. These findings are summarized in Fig. 12.

Slightly larger variations occur in the maximum value of $\bar{n}$ after 1 day, taken across the full range of the simulated initial cell densities (for example, when c_{tissue} is varied within the physiological range of 4–6%, the maximum value of $\bar{n}$ varies by up to 5.1% of its value at $c_{\text{tissue}} = 5\%$). When $c_{\text{tissue}} = 5\%$, reflecting the positive effect that increased oxygen availability has on cell survival (Fig. 13B).

However, within the approximated physiological range for c_{tissue} , the initial cell density at which this maximum is achieved, $\arg \max_{n_0}(\bar{n})$, alters by up to only 2.2% of its value at 5%, and by up to 13.4% across the entire range of simulated values of c_{tissue} . This suggests that the model's predictions for the optimal n_0 values to achieve the highest possible average cell density after 1 day are robust to potential changes in tissue oxygen concentration or error in the set value of c_{tissue} .

The concentration of VEGF in the surrounding tissue, v_{tissue} , was also varied from 0 to 1000 pg/ml. However, this had no discernible effect on the values $\arg \max_{n_0}(\bar{v})$ and $\arg \max_{n_0}(v_{SD})$ or the corresponding maximum values of $\bar{v}$ and v_{SD} (Fig. 13). This is likely due to the difference in the order of magnitude between the physiological boundary condition values and the VEGF concentrations generated by the cells within the construct. As expected, varying the value of v_{tissue} did not result in any changes in the simulation values of n as the viable cell governing equation is independent of VEGF concentration. This motivates the use of $v_{\text{tissue}} = 0$ ng/ml as the boundary condition.

3.1.6 Conclusion

In summary, Sect. 3 presents a multidisciplinary approach in three stages: 1. in silico model, which captures the physics and chemistry involved in the system; 2. in vitro experiments to parameterize the model, and 3. extending the parameterized model to a 3D environment and to make predictions relevant to NRC design. At every stage the predictions made were tested through sensitivity analysis.

The continuum model of cell-solute interactions applied to the in vitro scenario captured the overall trends observed in counterpart experimental data. However, model predictions compared to cell density data achieved a higher R^2 value than that for the VEGF data, indicating a closer match between model and experiment. This is hypothesized to be due to the VEGF governing equation containing a greater number of parameters with unknown values than the oxygen and cell governing equations.

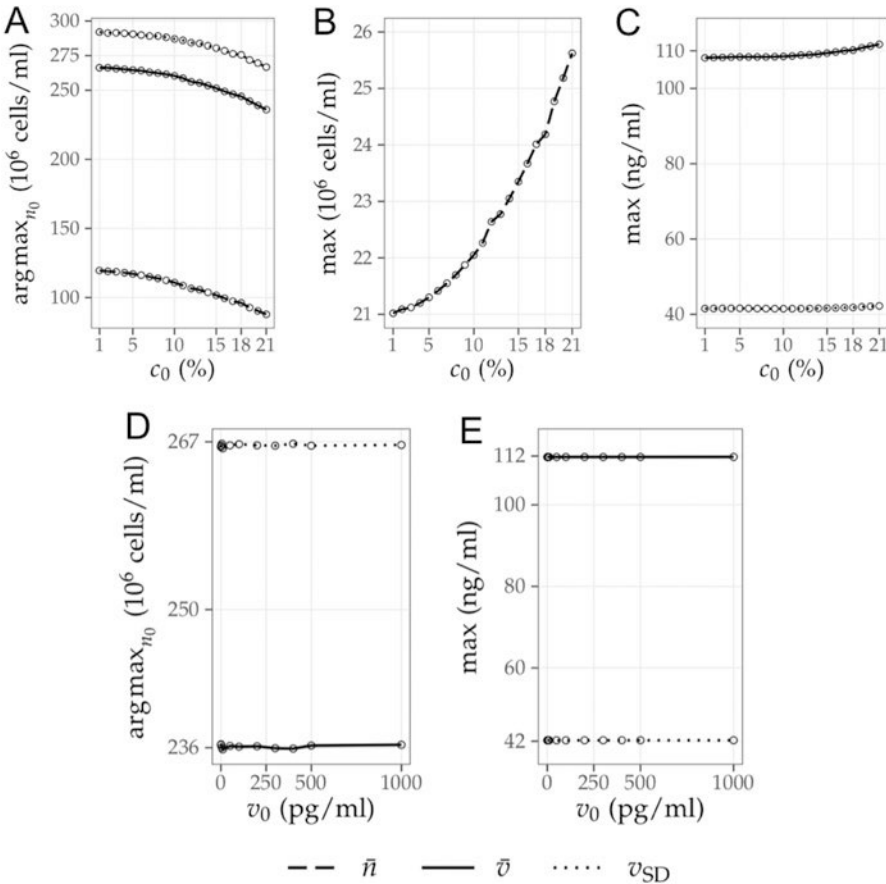


Fig. 12 Impact of c_0 upon 1 day simulation results; empty circles denotes the values of c_0 used to run the simulations. (A) Varying c_0 from 1% to 21% progressively reduces the values of n_0 that produce the maximums of $\bar{n}$, $\bar{v}$, and v_{SD} after 1 day. (B) The maximum value of $\bar{n}$ after 1 day, over all n_0 values, varies from 21×10^6 cells/ml when $c_0 = 1\%$ to 25.6×10^6 cells/ml when $c_0 = 21\%$. (C) The maximum values of $\bar{v}$ and v_{SD} after 1 day over all n_0 values increase only slightly as c_0 increase from 1% to 10%. Impact of v_0 upon 1 day simulation results; empty circles demote the values of v_0 used to run the simulations. (D) Varying v_0 up to 1000 pg/ml results in changes in the value of $\text{argmax}_{n_0}(\bar{v})$ and $\text{argmax}_{n_0}(v_{SD})$ after 1 day of less than 1% of their values when $v_0 = 0$. (E) The maximum values of $\bar{v}$ and v_{SD} also change by less than 1% as v_0 is varied from 0 to 1000 pg/ml

This made parameterization of the VEGF governing equation more difficult, and it was necessary to include two parameters that were explicit functions of the initial cell density n_0 in order to achieve a good fit. The cell death rate δ was also defined as a linear function of n_0 .

This illustrates the trade-off between model complexity, parameterization and reliability of predictions. The sensitivity analyses also demonstrated that VEGF predictions are more sensitive to changes in parameters values than the cell viability

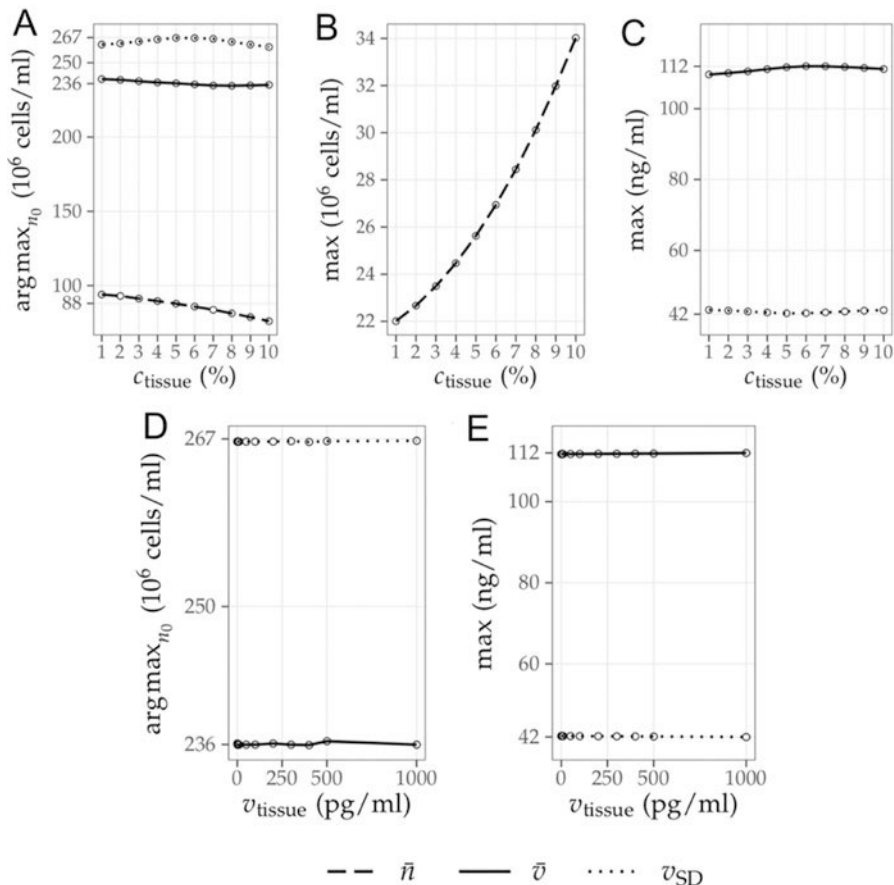


Fig. 13 Impact of c_{tissue} upon 1 day simulation results; empty circles denote the values of c_{tissue} used to run the simulations. (A) Varying c_{tissue} from the approximated physiological value of 5% results in only minor variations in the values of $\arg \max_{n_0}(\bar{v})$ and $\arg \max_{n_0}(v_{SD})$ after 1 day. When $c_{\text{tissue}} = 10\%$, the value of $\arg \max_{n_0}(\bar{n})$ is 13% less than its value when $c_{\text{tissue}} = 5\%$. (B) The maximum value of $\bar{n}$ after 1 day over all n_0 values varies from 22×10^{-6} cells/ml when $c_{\text{tissue}} = 1\%$ to 34×10^{-6} cells/ml when $c_{\text{tissue}} = 10\%$. (C) The maximum values of $\bar{v}$ and v_{SD} after 1 day over all n_0 values vary by less than 3% of their values when $c_{\text{tissue}} = 5\%$. Impact of v_{tissue} upon 1 day simulation results; empty circles denote the values of v_{tissue} used to run the simulations. (D) Varying v_{tissue} up to 1000 pg/ml results in changes in the value of $\arg \max_{n_0}(\bar{v})$ and $\arg \max_{n_0}(v_{SD})$ after 1 day of less than 1% of their values when $v_{\text{tissue}} = 0$. (E) The maximum values of $\bar{v}$ and v_{SD} also change by less than 1% as v_{tissue} is varied from 0 to 1000 pg/ml

predictions (Fig. 11). This motivates the need for accurate measurements of tissue VEGF levels, as well as potential limitations of the existing model.

After parameterization the model was extended to an in vivo scenario. Model predictions indicate that cell survival can be improved by tuning the seeded cell densities and distributions, and that seeding more cells in NRCs does not necessarily

lead to higher densities of viable cells at later time points in vivo. This runs contrary to the reasoning previously used in many experimental studies, which either neglects to consider the effect of seeding cell densities and distributions entirely, or assumes that seeding more cells will necessarily result in a greater number of viable cells over time. In fact, using fewer cells in NRCs could result in better cell survival, while reducing waste of cells.

As shown in Fig. 10A, the model predicts that the optimal density for uniformly seeded cells to maximize viable cell density after 24 h is around 88×10^6 cells/ml. Interestingly, this falls close to the figure of 80×10^6 cells/ml found by Mosahebi et al. to produce on average the greatest length of axonal regeneration after 3 weeks in vivo (Mosahebi et al., 2001). Increasing the cell density beyond this showed a decrease in regeneration in the experimental study. Guénard et al. also produced similar experimental results, concluding that 120×10^6 cells/ml produced the greatest number of myelinated axons after 3 weeks, although in this case the luminal diameter was altered along with the seeding cell density (Guenard et al. 1992). This variability in cell seeding density is also observed when the initial conditions are varied, as presented in Fig. 12.

In conclusion, the simulation results presented here corroborate the hypothesis that seeding cell densities and distributions can be tailored to enhance cell survival within the first 24 h post-implantation and to increase the steepness of VEGF gradients across engineered tissue. Through this work it was demonstrated how a continuum modeling framework can be parameterized with in vitro data and used to predict the behavior of relevant in vivo scenarios. Next these predictions should be evaluated experimentally.

3.2 The Role of Material Cues in Accelerating Neurite Regrowth: A Discrete Approach

3.2.1 Introduction

It is well established that material and durotactic cues play an important role in guiding neurite growth after injury. Here we explore a specific scenario where high-stiffness phosphate glass fibers (PGFs) are embedded in EngNT, aligned in the proximal-distal stump direction, to provide a guidance cue to accelerate regrowth of neurites. This setup has been explored through in vitro and in vivo assessment (Kim et al. 2015), yet there remain open questions around the optimal number and size of fibers to maximize cues for regeneration while maintaining sufficient space for neurites to grow. Here we seek to address this balance using a discrete modeling approach, which captures the influence of the NRC scaffold on the movement of neurites within a 3D environment (Laranjeira et al. 2022) (see Fig. 14).

Building on the literature base described in Sect. 2, we develop a discrete mathematical model for neurite growth in response to the 3D environment captured in Fig. 14. To mimic a realistic in vivo testing scenario, thousands of neurites must be simulated over a long period of time (weeks), in a 3D geometry, which is much larger than an

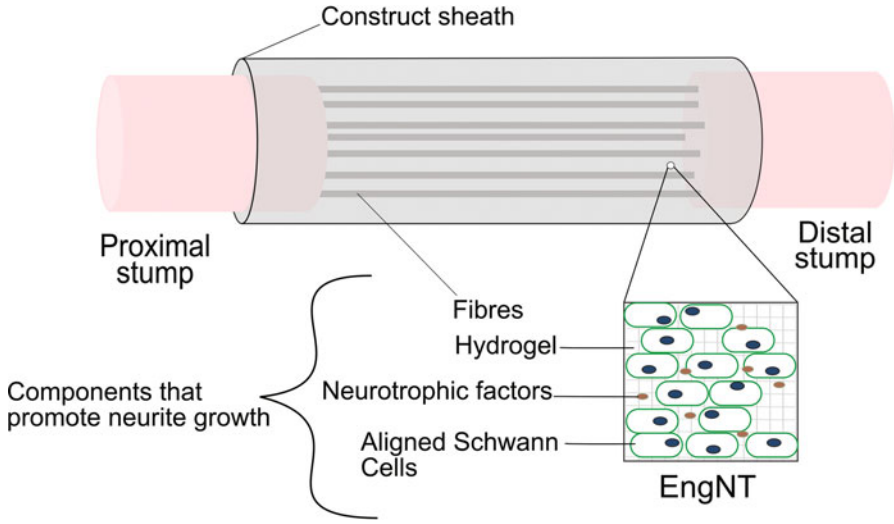


Fig. 14 Schematic, based on the work by Dodla and Bellamkonda (2008), of the NRC setup, consisting of EngNT with embedded phosphate glass fibers

individual neurite. From the outset, this requires careful consideration of the appropriate modeling framework that will minimize computational cost of the simulations.

We use a lattice-based random walk model to facilitate tracking individual neurites in a computationally tractable way. This approach also facilitates comparison with data from in vivo experiments, compromising neurite counts in proximal and distal cross-sections through the device, 8-weeks after a nerve repair. For demonstration purposes, we focus on exploring the role of the PGFs in providing stiffness cues to accelerate directed growth of neurites, although the model presented is sufficiently general to incorporate other cues in the future. The in-silico workflow encompasses four steps: model design, parameterization, optimization, and sensitivity analysis, described in the next sections.

3.2.2 Computational Model

The in silico model consists of two parts, the NRC geometry and modeling framework for describing movement of neurites within it. The former was developed using the software COMSOL Multiphysics, comprising a cylinder of length 13 mm and diameter 1.5 mm to mimic an NRC (see Fig. 15). This cylinder is discretized into nodal points, which serve as the potential neurite tip locations. PGFs are distributed within the NRC geometry at random, ensuring they do not overlap, by excluding these nodal points from the main domain (i.e., neurites cannot grow within a PGF). From experimental observations, we define an annular region, radius a , estimated to be approximately $20\mu\text{m}$, surrounding each fiber, which has a higher stiffness value (k_a) than the surrounding matrix. Additionally, it was experimentally observed that neurites within such a region grow at double the background rate, and we embed this

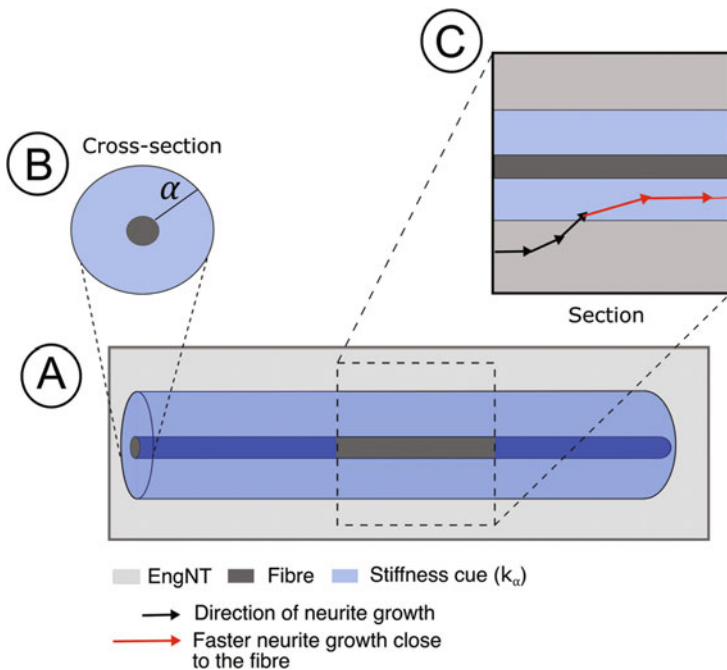


Fig. 15 Schematic representation of a PGF embedded in EngNT, (A) demonstrating the impact of stiffness cue k_a in an annular region, radius α , around each fiber. (B) As well as providing a directional bias when the neurites (black arrows) are within the volume occupied by the stiffness cue, (C) their growth approximately doubles (red arrows)

relationship in the mathematical model. The impact of PGFs on neurites is summarized in Fig. 15.

A number of neurites (N) are initiated at the proximal side of the cylinder. At every iteration of the model, the neurite tip samples the nodes within a region of $10 \pm \delta \mu\text{m}$ around itself, where the parameter δ essentially smooths any directional bias. From these nodes the neurites choose one based on the probability P_N , which follows a similar formulation as the ones established by Segev and Ben-Jacob (2000) as:

$$P_N = M(\theta) + B(w) + D(k_w), \quad (30)$$

where, w is the node number ($w = 1 \dots Z$, where Z is the total number of nodes in the mesh). The probability of the neurite tip moving to a new node is dependent on three forces: (i) the mechanical resistance of neurites to bending, $M(\theta)$, (ii) the bias to move toward the distal section of the device due to chemotactic and durotactic biases present within the NRC, $B(w)$, and (iii) the bias of neurites to move toward areas of higher stiffness, $D(k_w)$.

Following Segev and Ben-Jacob (2000), the mechanical resistance term is described as a probability density function (PDF) based on the angle (θ) made

between the current neurite direction and any future direction. The PDF is, contrary to Segev and Ben-Jacob's (2000) work, informed by experimental data collected by Razetti et al. (2016). Additionally, in order to facilitate the comparison of this probability with the other components in Eq. (30) the PDF is normalized.

To the best of our knowledge, there are no clear data to inform the bias terms $B(w)$ and $D(k_w)$ in the literature. We represent chemotactic and durotactic biases within the NRC as a constant bias of neurite tips moving in the proximal-distal direction (β_μ), without a counterpart value defined to represent the equivalent cues in an empty tube (β_ν). These two constants are fitted to data on neurite counts from in vivo experiments, which compare an empty tube to an EngNT construct from Georgiou et al. (2013). Validation of these bias values is presented in Sect. 3.2.3.

Finally, $D(k_w)$ (with k_w representing the stiffness value at each node) describes the bias of neurite growth toward stiffer regions. We extend the formulation of Segev and Ben-Jacob (2000), by prescribing that (a) neurite tips only respond to increases (and not decreases) in the stiffness field, and (b) $D(k_w)$ depends on the difference between the stiffness value at the neurite tip at time t , and sum of the stiffness values at all node options for the next time step (∇t). In summary,

$$D(k(w)) = \begin{cases} k(w_{t+\nabla t}) - k(w_t), & \text{if } k(w_t) \leq k(w_{t+\nabla t}) \\ 0, & \text{otherwise} \end{cases} \quad (31)$$

The impact of the stiffness field, and specifically value in the neighborhood surrounding individual fibers, is investigated in Sect. 3.2.5.

The random walk consists of finding the nodes that are at distance $10 \pm \delta \mu\text{m}$ from the current position of each neurite tip and calculating the probability associated to each of these nodes using Eq. (30). Then, for each neurite, a weighted cumulative sum is performed over possible next positions and used to inform, probabilistically, the next tip location (see Fig. 16). Following Segev and Ben-Jacob (2000), a noise reduction term is introduced to avoid nonphysiological neurite paths (e.g., the neurites should not form right angles with their previous location). The algorithm is also adapted to ensure that neurites do not occupy the same space, by removing nodes already occupied from the available domain.

Crucially, this modeling framework ensures that (a) the NRC geometry is accurately represented, (b) neurites grow in a physiologically realistic manner, and (c) neurites compete for space. The model includes numerous parameters that must be informed from biological data, as described in the next section.

3.2.3 Model Parameterization

All three terms in Eq. (30) require parameterization with biological data. The mechanical resistance terms are informed by Razetti et al. (2018), which consist of 3D data on neurite segments extracted from a drosophila model, and from which the angles between consecutive segments may be extracted (see Fig. 17). A probability density function (PDF) is fitted to this distribution and used to assign probabilities of movement.

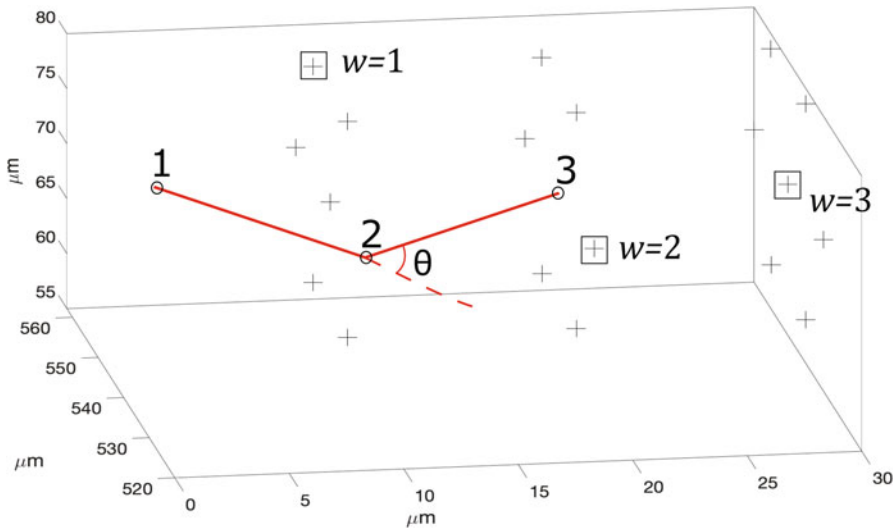
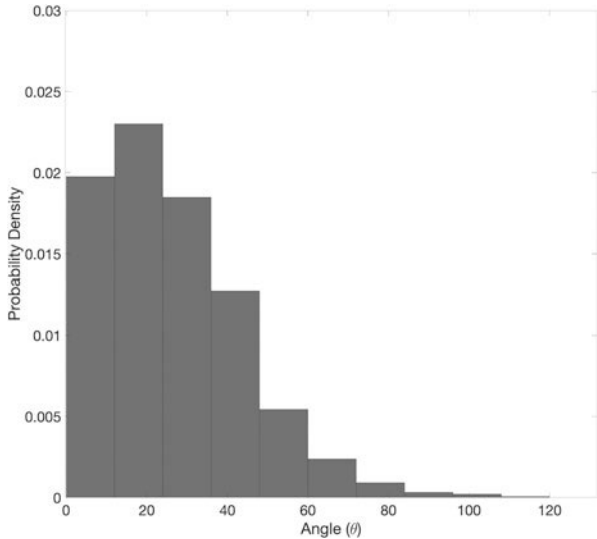


Fig. 16 Schematic of a neurite tip starting at surface number 1, progressing to surface number 3 after 2 iterations. Here a surface represents a cross-section through the NRC, including all the nodes in that plane. A neurite tip at surface 3 has the nodes (w) to choose from for the next iteration, and the choice of this next position is informed by the PDF $M(\theta_w)$ (a function of the angle (θ) made between segments)

Fig. 17 The neurite angle distribution made between neurite segments measured by Razetti et al. (2018)



The parameters that define the bias term $B(w)$ (β_μ , β_V , and the N_r) need informing from data. Georgiou et al. (2013) extracted neurite counts within an empty tube and EngNT-filled NRC, at four cross-sections along the proximal-distal distance

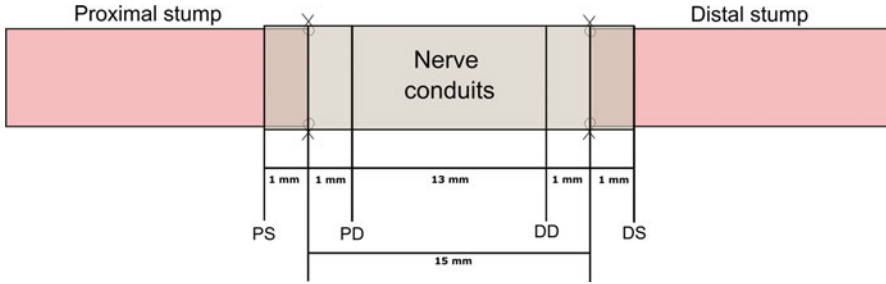


Fig. 18 Cross-sections where neurite count data were collected in Georgiou et al. (2013): 1 mm into the proximal (PS) and distal stump (DS) and 1 mm into the NRC from the proximal (PD) and distal (DD) stumps

(see Fig. 18). For example, based on 2000 neurites counted at the proximal stump, their data showed a drop of 90%, 30% in neurite number from the proximal to the distal end of injury side, for the empty tube and EngNT, respectively. A PSO algorithm was used to efficiently explore the parameter space for (β_μ and β_ν) by minimizing the error between the model simulations and the neurite count data for both the empty tube and EngNT. Simulation data indicate that the parameter β_μ tended to 1 in all simulations, after hundreds of iterations of the PSO model the 30 particles fitted the parameter to 1 with no variability. Hence, for the remaining analysis, β_μ is held fixed. The stopping criteria for the PSO was defined as when all the particles' result varied by less than 5%, and results of the data fit are shown in Fig. 19.

Finally, the $D(k_w)$ term from Eq. (30) represents the bias of neurites to move toward areas of higher stiffness, $D(k_w)$. Two parameters need to be characterized, the stiffness value in the boundary region surrounding each PGF, and the size of that boundary region. Georgiou et al. (2013) measure the radius of the boundary as $20\ \mu\text{m}$, whereas there are no data to inform the stiffness value of the EngNT. It is here assumed that the stiffness value for the nodes within the boundary (k_a) are one order of magnitude larger than the remaining, ensuring that neurites stay close to the fibers after they find them.

3.2.4 Optimization of PGF Number and Radius to Accelerate Neurite Regrowth

Here, again, a PSO algorithm is used to identify the radius and number of PGFs embedded within an NRC to promote the growth of 2,000 neurites toward the distal stump for 8-week simulations. The ranges for the two parameters optimized were defined as: (a) the radius being between $15\ \mu\text{m}$, the minimum possible for fabrication, and $750\ \mu\text{m}$, the radius of the NRC, (b) the number between 0 and 14,000.

Two examples of simulations of the growth of 300 neurites for 2 days within an NRC ($200\ \mu\text{m}$ in length) with different numbers of embedded fibers are presented in Fig. 20 (11 (Fig. 20A–C) and 300 (Fig. 20D–F)). From these simulations it can be clearly seen the impact of providing additional directional cues to neurites as, after 2 days, the

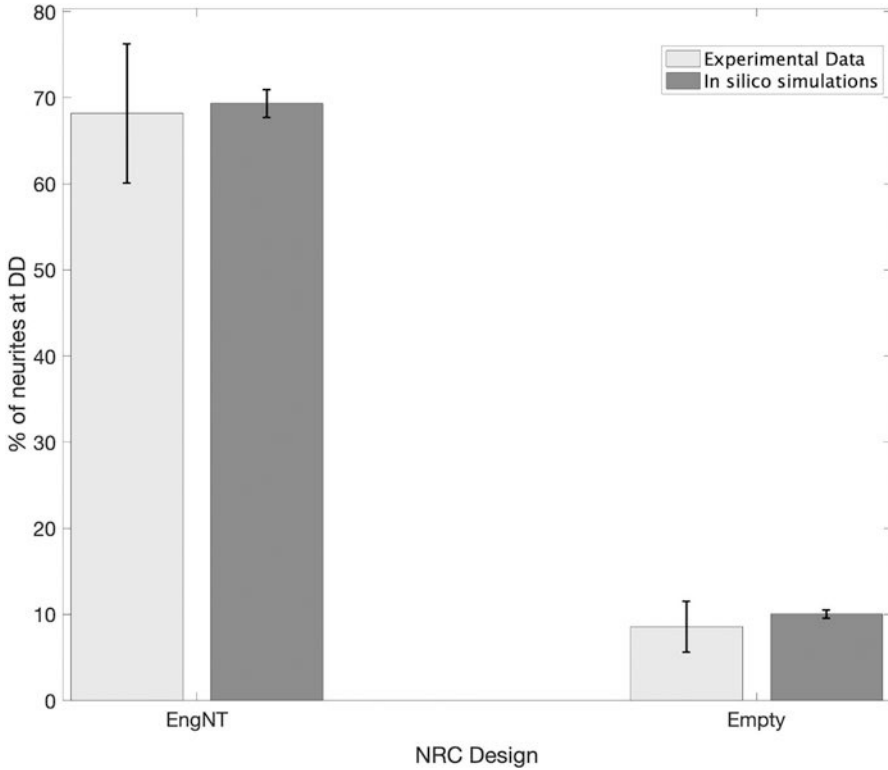


Fig. 19 Results from fitting the model parameters (β_μ , β_V and N_r) to data consisting of neurite counts from (Georgiou 2013) at the location PD and DD as seen in Fig. 18. For simplification here the counts are presented as percentage ratios between the proximal and the distal counts. The original neurite counts are presented as the light gray bars and the model simulations are presented as dark gray. The bars represent the mean of the measurements and simulations and the whiskers the standard deviation of both

percentage of neurites that made it to the distal side of the NRC increased from 10% to 20% to, approximately, 90% from the 11 to the 300 fibers simulation, respectively.

The output of the PSO algorithm identified that the best NRC design consisted in packing between 500 and 700 PGFs with $15\mu\text{m}$ radius within EngNT. This arrangement is predicted to achieve around 91–95% of neurites reaching the distal stump within 8 weeks.

3.2.5 Sensitivity Analysis to Inform Design Features

We employ the PAWN method described in Sect. 2.3 to test the sensitivity of the predictions of the model to the parameters that were defined in Sect. 3.2.4, including the forward bias (β_f), noise reduction parameter (N_r) as well as the stiffness (k_α) and the radius (α) of the high-stiffness zone surround the PGFs, and finally the standard

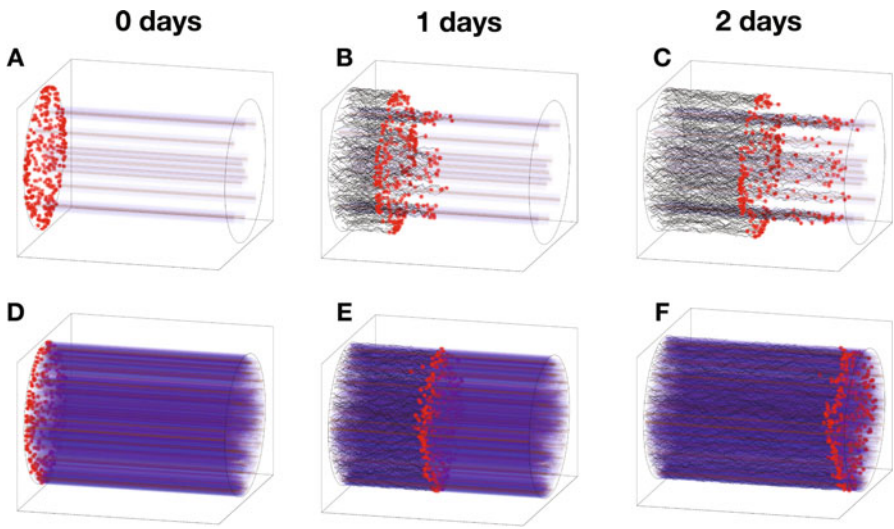


Fig. 20 Model predictions of 300 neurites growing within an NRC for 4 days. Two different numbers of PGFs embedded in EngNT were tested, 11 (top three images) and 300 (bottom three images). Three time points are captured 0 days (A, D), 1 day (B, E), and 2 days (C, F)

Table 3 Ranges used to perform the PAWN analysis

Parameter	Meaning	Range
β_v	Forward bias of neurites in an empty tube	$\pm 20\%$ of the value fitted in Sect. 3.2.4
N_r	Noise reduction parameter	$\pm 20\%$ of the value fitted in Sect. 3.2.4
k_α	Stiffness ratio between region surrounding a PGF and the remaining EngNT	1–3 [dimensionless]
α	Radius of the stiffer region surrounding the PGFs	10–50 μm
δ	Standard deviation of the growth rate.	$\pm 20\%$ of the current value

deviation attributed to the growth rate of neurites (δ). Assumed ranges for each parameter are summarized in Table 3.

The PAWN method was implemented to quantify the impact of changing parameters as presented in Fig. 19 on the prediction of model that approximately 650 fibers with a radius of 15 μm should be embedded. The output of the PAWN algorithm can be seen in Fig. 21 and it demonstrates that the most important parameters to ensure the predictability of the model inform neurite movement, the noise reduction term (N_r), and the standard deviation of the growth rate (δ). The importance of these two parameters is expected, since, the metric being optimized by the model is the distance traveled by the neurites, then it is rational that the choice of growth rate will lead to drastic changes in that measure.

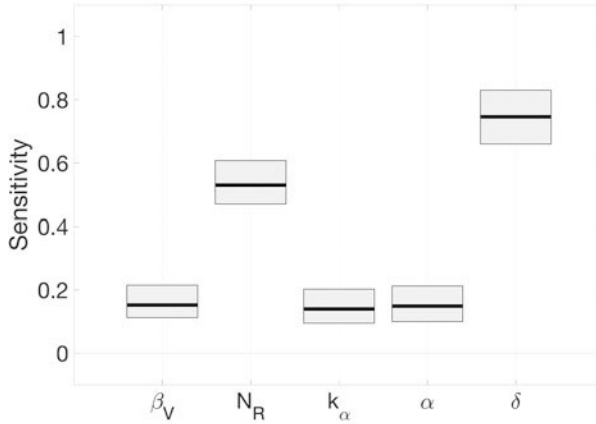


Fig. 21 Results from the PAWN analysis of the model, where simulations were performed within the ranges presented in Table 3: presents the outcome of the PAWN analysis quantifying the impact of each parameter on the output of the model

3.2.6 Conclusions

A computational model that simulates the role of biomaterial cues in guiding neurite regrowth in an NRC has been developed, and applied to a specific case study of optimizing the number and size of PGFs in an NRC. This model was discrete in nature, tracking individual neurites both spatially and in time.

Parameterization of the model was achieved via a PSO algorithm, focusing on the parameters that describe the response of regenerating neurites to underlying stiffness cues, and using in vivo data on neurite counts at different cross-sections through an NRC. Model outputs were able to predict the experimental data well; however, it was found that the efficacy of the parameterization is sensitive to small changes in parameters, a variability smaller than 0.5% results in changes in a mean error of $4.7 \pm 2.8\%$. Further experimental data (for example, at different cross-sections through the NRC) would aid in better informing this process.

A PSO algorithm was also used for the optimization step to inform the optimal number and radius of PGFs in the NRC. This outputted a PGF radius of $15\mu\text{m}$ and number between 500 and 700, with a maximum efficacy of 94%. In order to understand the dependency of the predictions of the model on the model parameter values, the PAWN methodology developed by Puy et al. (2019) was implemented. Three parameters were found to not having determinant effects on the output of the model, which include the forward bias β_v and those associated with the stiffness cues surrounding the PGFs (α and k_N). The noise reduction term N_R is important, underlying the need to uncover the cellular mechanisms responsible for cell motility in response to durotactic cues (and embed these relationships directly in the model). The parameter with largest relevance to the model predictions is the growth rate standard deviation δ , which will establish how many nodes each neurite can detect and choose from. Decreasing δ will impact the model predictions

in two ways: 1. neurites will move at a slower rate and 2. the neurites have less space to move in the NRC, which clarifies why it is such an important determinant of model outputs.

These results demonstrate the potential of a multidisciplinary approach to guide design of NRCs. It is essential for model development to happen in tandem with experimental research, with ongoing iteration between model and experiment. For example, the next step here should be to validate the predictions around PGF numbers and radius, and use the experimental findings to inform further model development. Such approaches are facilitated by (and, indeed, necessitate) genuinely multidisciplinary research teams where mathematicians and experimentalists work together on common research goals.

References

- Allena R, Scianna M, Preziosi L (2016) A cellular Potts model of single cell migration in presence of durotaxis. *Math Biosci* 275:57–70
- Anderson AR, Chaplain MAJ (1998) Continuous and discrete mathematical models of tumor-induced angiogenesis. *Bull Math Biol* 60:857–899
- Angius D, Wang H, Spinner RJ, Gutierrez-Cotto Y, Yaszemski MJ, Windebank AJ (2012) A systematic review of animal models used to study nerve regeneration in tissue-engineered scaffolds. *Biomaterials* 33:8034–8039. <https://doi.org/10.1016/j.biomaterials.2012.07.056>
- Aubert M, Chaplain MAJ, McDougall SR, Devlin A, Mitchell CA (2011) A continuum mathematical model of the developing murine retinal vasculature. *Bull Math Biol* 73:2430–2451. <https://doi.org/10.1007/s11538-011-9631-y>
- Azuaje F (2011) Computational discrete models of tissue growth and regeneration. *Brief Bioinform* 12:64–77. <https://doi.org/10.1093/bib/bbq017>
- Barkefors I, Le Jan S, Jakobsson L, Hejll E, Carlson G, Johansson H, Jarvius J, Park JW, Jeon NL, Kreuger J (2008) Endothelial cell migration in stable gradients of vascular endothelial growth factor a and fibroblast growth factor 2 effects on chemotaxis and chemokinesis. *J Biol Chem* 283:13905–13912
- Baroni G, Tarantola S (2014) A general probabilistic Framework for uncertainty and global sensitivity analysis of deterministic models: a hydrological case study. *Environ Model Softw* 51:26–34
- Boas SEM, Jiang Y, Merks RMH, Prokopiou SA, Rens EG (2018) Cellular Potts model: applications to vasculogenesis and angiogenesis. In: Louis P-Y, Nardi FR (eds) *Probabilistic cellular automata: theory, applications and future perspectives, emergence, complexity and computation*. Springer International Publishing, Cham, pp 279–310. https://doi.org/10.1007/978-3-319-65558-1_18
- Borgonovo E (2011) Sensitivity analysis in decision making. In: *Wiley encyclopedia of operations research and management science*. pp 1–11
- Bower JM, Beeman D (2012) *The book of GENESIS: exploring realistic neural models with the GEneral NEural Simulation System*. Springer Science & Business Media
- Boyd JG, Gordon T (2003) Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. *Mol Neurobiol* 27:277–324. <https://doi.org/10.1385/MN:27:3:277>
- Breward CJW, Byrne HM, Lewis CE (2002) The role of cell-cell interactions in a two-phase model for avascular tumour growth. *J Math Biol* 45:125–152. <https://doi.org/10.1007/s002850200149>
- Brown KS, Sethna JP (2003) Statistical mechanical approaches to models with many poorly known parameters. *Phys Rev E* 68:021904

- Burova I, Wall I, Shipley RJ (2019) Mathematical and computational models for bone tissue engineering in bioreactor systems. *J Tissue Eng* 10:204173141982792. <https://doi.org/10.1177/2041731419827922>
- Butt R (2009) Introduction to numerical analysis using MATLAB®. Jones & Bartlett Learning
- Cattin A-L, Burden JJ, Van Emmenis L, Mackenzie FE, Hoving JJA, Garcia Calavia N, Guo Y, McLaughlin M, Rosenberg LH, Quereda V, Jamecna D, Napoli I, Parrinello S, Enver T, Ruhrberg C, Lloyd AC (2015) Macrophage-induced blood vessels guide Schwann cell-mediated regeneration of peripheral nerves. *Cell* 162:1127–1139. <https://doi.org/10.1016/j.cell.2015.07.021>
- Chung AM (2018) Calcitonin gene-related peptide (CGRP): role in peripheral nerve regeneration. *Rev Neurosci* 29:369–376. <https://doi.org/10.1515/revneuro-2017-0060>
- Cickovski T, Aras K, Swat M, Merks RMH, Glimm T, Hentschel HGE, Alber MS, Glazier JA, Newman SA, Izaguirre JA (2007) From genes to organisms via the cell: a problem-solving environment for multicellular development. *Comput Sci Eng* 9:50–60. <https://doi.org/10.1109/MCSE.2007.74>
- Coelho RC, da Fontoura Costa L (1997) Morphologically realistic neural networks. In: Proceedings of the third IEEE international conference on engineering of complex computer systems (Cat. No. 97TB100168). IEEE, pp. 223–228
- Coffey W, Kalmykov YP (2012) The Langevin equation: with applications to stochastic problems in physics, chemistry and electrical engineering. World Scientific
- Coy RH (2020) Modelling the impact of cell seeding strategies on cell survival and vascularisation in engineered tissue. PhD Thesis, UCL (University College London)
- Coy R, Al-Badri G, Kayal C, O'Rourke C, Kingham PJ, Phillips JB, Shipley RJ (2020) Combining *in silico* and *in vitro* models to inform cell seeding strategies in tissue engineering. *J R Soc Interface* 17:20190801. <https://doi.org/10.1098/rsif.2019.0801>
- Croll TI, Gentz S, Mueller K, Davidson M, O'Connor AJ, Stevens GW, Cooper-White JJ (2005) Modelling oxygen diffusion and cell growth in a porous, vascularising scaffold for soft tissue engineering applications. *Chem Eng Sci* 60:4924–4934. <https://doi.org/10.1016/j.ces.2005.03.051>
- Daly W, Yao L, Zeugolis D, Windebank A, Pandit A (2012) A biomaterials approach to peripheral nerve regeneration: bridging the peripheral nerve gap and enhancing functional recovery. *J R Soc Interface* 9:202–221. <https://doi.org/10.1098/rsif.2011.0438>
- Deumens R, Bozkurt A, Meek MF, Marcus MAE, Joosten EAJ, Weis J, Brook GA (2010) Repairing injured peripheral nerves: bridging the gap. *Prog Neurobiol* 92:245–276. <https://doi.org/10.1016/j.pneurobio.2010.10.002>
- Dickinson RB, Tranquillo RT (1993) A stochastic model for adhesion-mediated cell random motility and haptotaxis. *J Math Biol* 31:563–600
- Dodla MC, Bellamkonda RV (2008) Peripheral nerve regeneration. In: Principles of regenerative medicine. Elsevier, pp 1270–1285. <https://doi.org/10.1016/B978-012369410-2.50076-0>
- Dodla MC, Alvarado-Velez M, Mukhatyar VJ, Bellamkonda RV (2019) Chapter 69 peripheral nerve regeneration. In: Atala A, Lanza R, Mikos AG, Nerem R (eds) Principles of regenerative medicine (third edition). Academic Press, Boston, pp 1223–1236. <https://doi.org/10.1016/B978-0-12-809880-6.00069-2>
- Doob JL (1942) The Brownian movement and stochastic equations. *Ann Math* 43:351–369
- Dyson F (2004) A meeting with Enrico Fermi. *Nature* 427:297–297. <https://doi.org/10.1038/427297a>
- Eberhart R, Kennedy J (1995) Particle swarm optimization. In: Proceedings of the IEEE international conference on neural networks. Citeseer, pp 1942–1948
- Faroni A, Mobasser SA, Kingham PJ, Reid AJ (2015) Peripheral nerve regeneration: Experimental strategies and future perspectives. *Adv Drug Deliv Rev* 82–83:160–167. <https://doi.org/10.1016/j.addr.2014.11.010>
- Fontana X, Hristova M, Da Costa C, Patodia S, Thei L, Makwana M, Spencer-Dene B, Latouche M, Mirsky R, Jessen KR, Klein R, Raivich G, Behrens A (2012) c-Jun in Schwann cells promotes axonal regeneration and motoneuron survival via paracrine signaling. *J. Cell Biol* 198:127–141. <https://doi.org/10.1083/jcb.201205025>

- Frostick SP, Yin Q, Kemp GJ (1998) Schwann cells, neurotrophic factors, and peripheral nerve regeneration. *Microsurgery* 18:397–405
- Georgiou M (2013) Development of a tissue engineered implantable device for the surgical repair of the peripheral nervous system. <https://doi.org/10.21954/OU.RO.0000EEF7>
- Georgiou M, Bunting SCJ, Davies HA, Loughlin AJ, Golding JP, Phillips JB (2013) Engineered neural tissue for peripheral nerve repair. *Biomaterials* 34:7335–7343. <https://doi.org/10.1016/j.biomaterials.2013.06.025>
- Grinsell D, Keating CP (2014) Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies [WWW Document]. *Biomed Res Int*. <https://doi.org/10.1155/2014/698256>
- Gu X, Ding F, Williams DF (2014) Neural tissue engineering options for peripheral nerve regeneration. *Biomaterials* 35:6143–6156. <https://doi.org/10.1016/j.biomaterials.2014.04.064>
- Guenard V, Kleitman N, Morrissey T, Bunge R, Aebischer P (1992) Syngeneic Schwann cells derived from adult nerves seeded in semipermeable guidance channels enhance peripheral nerve regeneration. *J Neurosci* 12:3310–3320. <https://doi.org/10.1523/JNEUROSCI.12-09-03310.1992>
- Guidolin D, Albertin G, Sorato E, Oselladore B, Mascarin A, Ribatti D (2009) Mathematical modeling of the capillary-like pattern generated by adrenomedullin-treated human vascular endothelial cells *in vitro*. *Dev Dyn* 238:1951–1963. <https://doi.org/10.1002/dvdy.22022>
- Gutenkunst RN, Waterfall JJ, Casey FP, Brown KS, Myers CR, Sethna JP (2007) Universally Sloppy parameter sensitivities in systems biology models. *PLoS Comput Biol* 3:e189
- Hines ML, Carnevale NT (1997) The NEURON simulation environment. *Neural Comput* 9:1179–1209
- Hirashima T, Rens EG, Merks RMH (2017) Cellular Potts modeling of complex multicellular behaviors in tissue morphogenesis. *Develop Growth Differ* 59:329–339. <https://doi.org/10.1111/dgd.12358>
- Iooss B, Lemaître P (2015) A review on global sensitivity analysis methods. In: *Uncertainty management in simulation-optimization of complex systems*. Springer, pp 101–122
- Jessen KR, Mirsky R (2016) The repair Schwann cell and its function in regenerating nerves. *J Physiol* 594:3521–3531. <https://doi.org/10.1113/JP270874>
- Jurecka W, Ammerer HP, Lassmann H (1975) Regeneration of a transected peripheral nerve. An autoradiographic and electron microscopic study. *Acta Neuropathol (Berl)* 32:299–312
- Kaplan HM, Mishra P, Kohn J (2015) The overwhelming use of rat models in nerve regeneration research may compromise designs of nerve guidance conduits for humans. *J Mater Sci Mater Med* 26:226. <https://doi.org/10.1007/s10856-015-5558-4>
- Kim Y-P, Lee G-S, Kim J-W, Kim MS, Ahn H-S, Lim J-Y, Kim H-W, Son Y-J, Knowles JC, Hyun JK (2015) Phosphate glass fibres promote neurite outgrowth and early regeneration in a peripheral nerve injury model. *J Tissue Eng Regen Med* 9:236–246
- Krause AL, Beliaev D, Van Gorder RA, Waters SL (2017) Lattice and continuum modelling of a bioactive porous tissue scaffold. *ArXiv Prepr. ArXiv170207711*
- Lafosse A, Dufey C, Beauloye C, Horman S, Dufrane D (2016) Impact of hyperglycemia and low oxygen tension on adipose-derived stem cells compared with dermal fibroblasts and keratinocytes: importance for wound healing in Type 2 diabetes. *PLoS One* 11:e0168058
- Laranjeira S, Pellegrino G, Bhangra KS, Phillips JB, Shipley RJ (2022) In silico framework to inform the design of repair constructs for peripheral nerve injury repair. *J R Soc Interface* 20210824. <https://doi.org/10.1098/rsif.2021.0824>
- Lewis MC, Macarthur BD, Malda J, Pettet G, Please CP (2005) Heterogeneous proliferation within engineered cartilaginous tissue: the role of oxygen tension. *Biotechnol Bioeng* 91:607–615. <https://doi.org/10.1002/bit.20508>
- Liu H, Chen W, Sudjianto A (2006) Relative entropy based method for probabilistic sensitivity analysis in engineering design. *J Mech Des* 128:326–336
- Lundborg G (2003) Richard P. Bunge memorial lecture. Nerve injury and repair – a challenge to the plastic brain. *J Peripher Nerv Syst* 8:209–226
- Martens W, Sanen K, Georgiou M, Struys T, Bronckaers A, Ameloot M, Phillips J, Lambrichts I (2014) Human dental pulp stem cells can differentiate into Schwann cells and promote and

- guide neurite outgrowth in an aligned tissue-engineered collagen construct *in vitro*. *FASEB J* 28: 1634–1643. <https://doi.org/10.1096/fj.13-243980>
- McDougall SR, Anderson ARA, Chaplain MAJ, Sherratt JA (2002) Mathematical modelling of flow through vascular networks: implications for tumour-induced angiogenesis and chemotherapy strategies. *Bull Math Biol* 64:673–702. <https://doi.org/10.1006/bulm.2002.0293>
- McDougall SR, Anderson AR, Chaplain MA (2006) Mathematical modelling of dynamic adaptive tumour-induced angiogenesis: clinical implications and therapeutic targeting strategies. *J Theor Biol* 241:564–589
- McDougall SR, Watson MG, Devlin AH, Mitchell CA, Chaplain MAJ (2012) A hybrid discrete-continuum mathematical model of pattern prediction in the developing retinal vasculature. *Bull Math Biol* 74:2272–2314. <https://doi.org/10.1007/s11538-012-9754-9>
- Merks RMH, Brodsky SV, Goligorsky MS, Newman SA, Glazier JA (2006) Cell elongation is key to *in silico* replication of *in vitro* vasculogenesis and subsequent remodeling. *Dev Biol* 289:44–54. <https://doi.org/10.1016/j.ydbio.2005.10.003>
- Metzcar J, Wang Y, Heiland R, Macklin P (2019) A review of cell-based computational modeling in cancer biology. *JCO Clin Cancer Inform* 3:1–13. <https://doi.org/10.1200/CCI.18.00069>
- Mosahebi A, Woodward B, Wiberg M, Martin R, Terenghi G (2001) Retroviral labeling of Schwann cells: *in vitro* characterization and *in vivo* transplantation to improve peripheral nerve regeneration. *Glia* 34:8–17
- Muheremu A, Ao Q (2015) Past, present, and future of nerve conduits in the treatment of peripheral nerve injury [WWW Document]. *Biomed Res Int*. <https://doi.org/10.1155/2015/237507>
- Napoli I, Noon LA, Ribeiro S, Kerai AP, Parrinello S, Rosenberg LH, Collins MJ, Harris Singh MC, White IJ, Woodhoo A, Lloyd AC (2012) A central role for the ERK-signaling pathway in controlling Schwann cell plasticity and peripheral nerve regeneration *in vivo*. *Neuron* 73:729–742. <https://doi.org/10.1016/j.neuron.2011.11.031>
- Nectow AR, Marra KG, Kaplan DL (2012) Biomaterials for the development of peripheral nerve guidance conduits. *Tissue Eng Part B Rev* 18:40–50. <https://doi.org/10.1089/ten.TEB.2011.0240>
- Nguyen QT, Sanes JR, Lichtman JW (2002) Pre-existing pathways promote precise projection patterns. *Nat Neurosci* 5:861–867. <https://doi.org/10.1038/nn905>
- Novosel EC, Kleinhans C, Kluger PJ (2011) Vascularization is the key challenge in tissue engineering. *Adv Drug Deliv Rev* 63:300–311
- O’Dea R, Byrne H, Waters S (2012) Continuum modelling of *in vitro* tissue engineering: a review. In: Geris L (ed) Computational modeling in tissue engineering, studies in mechanobiology, tissue engineering and biomaterials. Springer, Berlin/Heidelberg, pp 229–266. https://doi.org/10.1007/8415_2012_140
- Odedra D, Chiu LL, Shoichet M, Radisic M (2011) Endothelial cells guided by immobilized gradients of vascular endothelial growth factor on porous collagen scaffolds. *Acta Biomater* 7:3027–3035
- Parrinello S, Napoli I, Ribeiro S, Wingfield Digby P, Fedorova M, Parkinson DB, Doddrell RDS, Nakayama M, Adams RH, Lloyd AC (2010) EphB signaling directs peripheral nerve regeneration through Sox2-dependent Schwann cell sorting. *Cell* 143:145–155. <https://doi.org/10.1016/j.cell.2010.08.039>
- Patel NP, Lyon KA, Huang JH (2018) An update—tissue engineered nerve grafts for the repair of peripheral nerve injuries. *Neural Regen Res* 13:764
- Peripheral nerve graft – Mayo Clinic [WWW Document] (n.d.). <https://www.mayoclinic.org/diseases-conditions/peripheral-nerve-injuries/multimedia/img-20337149>. Accessed 3 Nov 2021
- Pianosi F, Wagener T (2015) A simple and efficient method for global sensitivity analysis based on cumulative distribution functions. *Environ Model Softw* 67:1–11
- Plischke E, Boronovo E, Smith CL (2013) Global sensitivity measures from given data. *Eur J Oper Res* 226:536–550
- Podhajsky RJ, Myers RR (1995) A diffusion-reaction model of nerve regeneration. *J Neurosci Methods* 60:79–88. [https://doi.org/10.1016/0165-0270\(94\)00222-3](https://doi.org/10.1016/0165-0270(94)00222-3)
- Poli R, Kennedy J, Blackwell T (2007) Particle swarm optimization. *Swarm Intell* 1:33–57

- Prokopiou SA, Owen MR, Byrne HM, Ziyad S, Domigan C, Iruela-Arispe ML, Jiang Y (2016) Integrative modeling of sprout formation in angiogenesis: coupling the VEGFA-Notch signaling in a dynamic stalk-tip cell selection. *ArXiv Prepr. ArXiv160602167*
- Puy A, Piano SL, Saltelli A (2019) A sensitivity analysis of the PAWN sensitivity index. *ArXiv Prepr. ArXiv190404488*
- Rabitz H (1989) Systems analysis at the molecular scale. *Science* 246:221–226
- Razetti A, Descombes X, Medioni C, Besse F (2016) A stochastic framework for neuronal morphological comparison: application to the study of IMP knockdown effects in drosophila gamma neurons. In: *International joint conference on biomedical engineering systems and technologies*. Springer, pp 145–166
- Razetti A, Medioni C, Malandain G, Besse F, Descombes X (2018) A stochastic framework to model axon interactions within growing neuronal populations. *PLoS Comput Biol* 14:e1006627
- Rutkowski GE, Heath CA (2002) Development of a bioartificial nerve graft. II. Nerve regeneration *in vitro*. *Biotechnol Prog* 18:373–379. <https://doi.org/10.1021/bp020280h>
- Saltelli A, Tarantola S, Chan K-S (1999) A quantitative model-independent method for global sensitivity analysis of model output. *Technometrics* 41:39–56
- Saltelli A, Tarantola S, Campolongo F, Ratto M (2002) *Sensitivity analysis in practice*. Wiley, Chichester. <https://doi.org/10.1002/0470870958>
- Saltelli A, Annoni P, Azzini I, Campolongo F, Ratto M, Tarantola S (2010) Variance based sensitivity analysis of model output. Design and estimator for the total sensitivity index. *Comput Phys Commun* 181:259–270
- Samsonovich AV, Ascoli GA (2005) Statistical determinants of dendritic morphology in hippocampal pyramidal neurons: a hidden Markov model. *Hippocampus* 15:166–183
- Schienbein M, Gruler H (1993) Langevin equation, Fokker-Planck equation and cell migration. *Bull Math Biol* 55:585–608
- Segev R, Ben-Jacob E (2000) Generic modeling of chemotactic based self-wiring of neural networks. *Neural Netw* 13:185–199
- Smith JT, Tomfohr JK, Wells MC, Beebe TP, Kepler TB, Reichert WM (2004) Measurement of cell migration on surface-bound fibronectin gradients. *Langmuir* 20:8279–8286
- Stefanoni F, Ventre M, Mollica F, Netti PA (2011) A numerical model for durotaxis. *J Theor Biol* 280:150–158. <https://doi.org/10.1016/j.jtbi.2011.04.001>
- Stokes CL, Lauffenburger DA (1991) Analysis of the roles of microvessel endothelial cell random motility and chemotaxis in angiogenesis. *J Theor Biol* 152:377–403
- Tarantola S, Becker W (2016) SIMLAB software for uncertainty and sensitivity analysis. In: Ghanem R, Higdon D, Owhadi H (eds) *Handbook of uncertainty quantification*. Springer International Publishing, Cham, pp 1–21. https://doi.org/10.1007/978-3-319-11259-6_61-1
- Tranquillo RT, Lauffenburger DA (1987) Stochastic model of leukocyte chemosensory movement. *J Math Biol* 25:229–262
- Van Pelt J, Dityatev AE, Uylings HB (1997) Natural variability in the number of dendritic segments: Model-based inferences about branching during neurite outgrowth. *J Comp Neurol* 387:325–340
- Vaz AIF, Vicente LN (2007) A particle swarm pattern search method for bound constrained global optimization. *J Glob Optim* 39:197–219
- Vokes SA, Yatskevych TA, Heimark RL, McMahon J, McMahon AP, Antin PB, Krieg PA (2004) Hedgehog signaling is essential for endothelial tube formation during vasculogenesis. *Development* 131:4371–4380. <https://doi.org/10.1242/dev.01304>
- Wang L, Sanford MT, Xin Z, Lin G, Lue TF (2015) Role of Schwann cells in the regeneration of penile and peripheral nerves. *Asian J Androl* 17:776–782. <https://doi.org/10.4103/1008-682X.154306>
- Ward JP, King JR (1997) Mathematical modelling of avascular-tumour growth. *IMA J Math Appl Med Biol* 14:39–69

- Wu JS, Luo L (2006) A protocol for mosaic analysis with a repressible cell marker (MARCM) in *Drosophila*. *Nat Protoc* 1:2583
- Wu P, Fu Y, Cai K (2014) Regulation of the migration of endothelial cells by a gradient density of vascular endothelial growth factor. *Colloids Surf B Biointerfaces* 123:181–190
- Zochodne DW (2008) *Neurobiology of peripheral nerve regeneration* by Douglas W. Zochodne [WWW Document]. Cambridge Core. <https://doi.org/10.1017/CBO9780511541759>
- Zubler F, Douglas R (2009) A framework for modeling the growth and development of neurons and networks. *Front Comput Neurosci* 3. <https://doi.org/10.3389/neuro.10.025.2009>

Part III

Biomaterials for Peripheral Nerve Regeneration



Biomaterials and Scaffolds for Repair of the Peripheral Nervous System

Caroline S. Taylor and John W. Haycock

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Abstract

Peripheral nerve injuries are a global challenge, causing long term disabilities to the patient and reducing quality of life. Such injuries are common and frequently require surgical intervention due to the complexity and significance of the injury. Autografts are the current clinical gold standard intervention, however, a major disadvantage exists with donor site morbidity and a lack of donor nerve tissue. Therefore, the use of nerve guide conduits (NGCs) is an attractive alternative approach to treat peripheral nerve injuries. Commercial NGCs are hollow tubes, and also wraps, manufactured from natural or synthetic materials. However, only modest success is reported with these devices, and therefore a need exists to create materials that are more beneficial for reinnervation, combined with more elegant methods for fabricating guidance structures. This chapter reviews current biomaterials used for peripheral nerve repair, both experimentally, clinically and commercially. We also discuss advances in the field, from hollow tube designs to those with complex structures, the use of intraluminal scaffolds, incorporation of growth factors and the use of autologous and allogenic supporting cells to improve nerve regeneration *in vivo*.

1 Introduction

The nervous system is the most complex structure in the human body and is divided into the central nervous system and the peripheral nervous system (Paxinos et al. 2012). The central nervous system consists of the brain, brain stem, and the spinal cord, and the peripheral nervous system includes the nerves and supporting cells that lie outside the central nervous system (Marani et al. 2012). Both work together continuously by responding to external stimuli, and transmitting electrical signals from the brain to the limbs and organs and back (Marani et al. 2012). Supporting cells of the central and peripheral nervous system, the oligodendrocytes and Schwann cells, are responsible for forming a myelin sheath, which aides in the salutatory conduction of electrical action potentials, in turn increasing conduction speed (Huxley and Stämpeli 1949). Oligodendrocytes myelinate several adjacent axons, whereas Schwann cells myelinate a single segment of an axon and incorporate themselves into the myelin sheath (Baumann and Pham-Dinh 2001; Lowe and Anderson 2015). Due to the complexity of the nervous system, injuries can be difficult to treat. However, injuries of the peripheral nervous system can be treated, depending on the severity of the injury and the treatment the patient receives.

2 Peripheral Nerve Injuries, Regeneration, and Current Treatments

2.1 Peripheral Nerve Injuries and Nerve Regeneration

Peripheral nerve injuries are a global challenge. In Europe alone, 300,000 people are affected by a peripheral nerve injury annually (Belkas et al. 2004). They reduce quality of life, causing long term disabilities for patients, with a concomitant cost to the economy and worldwide healthcare system every year (Nectow et al. 2012). The majority of peripheral nerve injuries arise from traumatic incidents, such as road traffic accidents and industrial accidents, but they can also arise from medical disorders and even childbirth (Pfister et al. 2011). Peripheral nerves have regeneration capabilities and can regenerate after injury at a rate of 1–3 mm per day (Deumens et al. 2010). This is via a process called Wallerian degeneration and axonal regeneration in which Schwann cells, the supporting cells of the peripheral nervous system, have a key role in nerve repair after injury (Perry et al. 1987). Macrophages infiltrate the injury site in which they clear away debris of the degenerating distal stump, as well as secreting pro-inflammatory cytokines such as IL-1 which stimulates Schwann cell proliferation (Deumens et al. 2010). The endoneurial tubes of the distal stump collapse, and Schwann cells, having undergone differentiation into Büngner Schwann cells, form the Bands of Büngner (Pfister et al. 2011). Büngner Schwann cells secrete neurotrophic factors, in particular brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF). Axons at the proximal stump of the nerve begin to regenerate and develop axonal sprouts (Deumens et al. 2010). The axons sprout across the injury site to the distal stump guided by the “Bands of Büngner” (Deumens et al. 2010). Over time, Büngner Schwann cells redifferentiate back to myelinating Schwann cells, to reform the myelin sheath of the new regenerating nerve (Pfister et al. 2011).

2.2 Current Treatments

Although repair and regeneration of peripheral nerves is possible after injury, often the axonal damage is too severe and therefore surgical intervention is required. The type of treatment, and surgical intervention, is dependent on the severity of the nerve injury and the size of the injury gap between the proximal and distal stumps of the damaged nerve (Pfister et al. 2011). Direct end-to-end suturing of the proximal and distal stumps of the nerve can be performed if the nerve is not under any tension, and the gap is less than 10 millimeters (Deumens et al. 2010). Regenerating axons grow from the proximal stump directly across the injury to the distal stump and continue to grow along the distal tract with the potential for reinnervation if reaching a target site (e.g., neuromuscular junction). Autografts are considered the “gold standard” for peripheral nerve repair, generally bridging larger gaps of over 20 mm using the patient’s own nerve. The sural nerve is usually the donor source, a sensory nerve removed surgically from the patient’s lower limb (Daly et al. 2012).

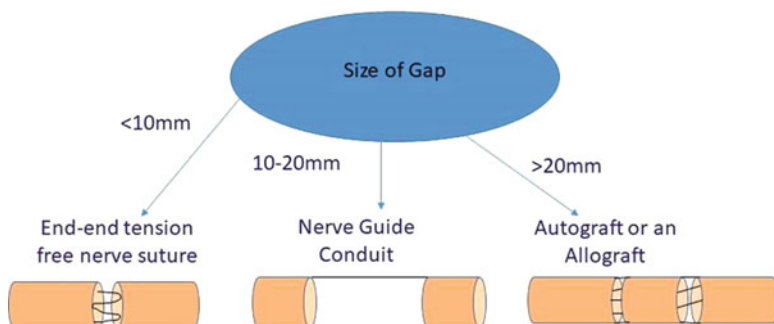


Fig. 1 The main approaches for repairing peripheral nerve gaps by injury length

However, autografts have a 50% success rate, and major disadvantages are associated with their use, including donor site morbidity, neuropathic pain, and neuroma formation, plus a lack of donor nerve when required for more extensive surgical reconstructions (Kehoe et al. 2012).

Allografts and xenografts provide an alternative choice of treatment for peripheral nerve repair, although they also present with disadvantages, such as the need for immunosuppressive drugs and the risk of zoonotic infection from xenografts (Arslantunali et al. 2014). Therefore, there is a clinical need for alternative grafting materials for peripheral nerve repair, and the use of materials such as biodegradable polymers is an attractive approach. Nerve guide conduits (NGCs) are currently used to bridge small nerve gaps of 10–20 mm (Pateman et al. 2015). These entubulation devices most often take the form of hollow tubes and cuffs connecting the ends of the proximal and distal stumps of the nerve, and are held in place with fibrin glue or sutures (Bell and Haycock 2012). Figure 1 summarizes the different surgical approaches to peripheral nerve repair.

There are currently 17 commercially available devices on the market, manufactured from both natural and synthetic materials (summarized in Table 1).

2.3 Nerve Guide Conduits Manufactured from Natural Materials

Naturally derived materials, and tissues, are used in devices for nerve repair. The company AxoGen manufactures a decellularized human nerve allograft (Avance[®] Nerve Graft) which is reported to bridge injury gaps of up to 20 mm (Kehoe et al. 2012). AxoGen also manufactures Axoguard[®] Nerve connector, an NGC protecting wrap comprised of porcine submucosa extracellular matrix (Bell and Haycock 2012). However, the limitations with the Avance[®] Nerve Graft are the amount of human donor nerve available, and increased risks of scarring and fibrosis “associated with host rejection” (Kehoe et al. 2012).

Collagen is a widely used natural material in peripheral nerve repair. As a component of the extracellular matrix (ECM), it is biocompatible, has similar mechanical properties to native nerve, and promotes cellular adhesion.

Table 1 FDA approved devices for treating peripheral nerve injury. (Adapted from Bell et al. and updated (Bell and Haycock 2012))

Commercial name	Material	Device type	Company manufactured by
NeuraGen®	Type I collagen	NGC	Integra Life Sciences Corp
NeuroMatrix™	Type I collagen	NGC	Collagen Matrix Inc.
Neuroflex™	Type I collagen	NGC	Collagen Matrix Inc.
Neurowrap™	Type I collagen	Wrap	Integra Life Sciences Corp
Neuromend™	Type I collagen	Wrap	Collagen Matrix Inc.
RevolNerve®	Type I and III collagen	NGC	Orthomed
Neuromaix®	Type I collagen	NGC	Matricel GmbH
Reaxon®	Chitosan	NGC	Medovent
AxoGuard™	Porcine small intestinal submucosa	Xenograft	AxoGen
Avance® Nerve Graft	Human nerve	Allograft	AxoGen
Neurotube®	Poly(glycolic Acid) (PGA)	NGC	Synovis® Micro Companies Alliance Inc.
Neurolac®	PolyDL-lactideε-caprolactone (PCL)	NGC	Polyganics B.V
SaluTunnel™	Salubria-biostable polyvinyl alcohol hydrogel	NGC	Salumedica
Salubridge™	Salubria-biostable polyvinyl alcohol hydrogel	Protectant nerve wrap device	Salumedica

Collagen type I is used to manufacture devices such as NeuraGen®, NeuroFlex™, NeuroMatrix™, NeuraWrap™, NeuroMend™, Neuromaix®, and RevolNerve® (Bell and Haycock 2012). The NeuraGen® tube is reported to bridge injury gaps up to 40 mm, with results comparable to autograft use. Type I collagen is also used by Matricel GmbH to manufacture a Neuromaix® nerve guide conduit (Bozkurt et al. 2017). The Neuromaix® NGC is the only conduit on the market to have an internal scaffold within the lumen of the tube in the form of a freeze dried collagen type I sponge (Bozkurt et al. 2014). Type I and III collagen are used to fabricate RevolNerve® and are manufactured by Orthomed (Bell and Haycock 2012; Luca et al. 2014). The first natural nerve guide conduit, not to be manufactured from collagen, is the Reaxon® Nerve Guide which is made from chitosan. This in the form of a hollow conduit, manufactured by Medovent, and has gained FDA approval (Neubrech et al. 2016). Chitosan is a biocompatible naturally derived biopolymer which exhibits good biocompatibility and mechanical properties similar to native nerve (Bak et al. 2017). Medovent claims that the Reaxon® Nerve Guide can bridge nerve gaps up to 26 mm and that results are comparable to autograft use (Boecker et al. 2019). However, issues have arisen using some naturally derived FDA approved conduits, such as the need for immunosuppressive drugs, batch to batch variability, and rapid degradation rate (Kehoe et al. 2012).

2.4 Nerve Guide Conduits Manufactured from Synthetic Materials

Synthetic materials are currently used to manufacture FDA approved nerve guide conduits. Salumedica have two nonresorbable devices manufactured for nerve repair. Salubridge™ is a protectant nerve wrap device, and SaluTunnel™ is a nerve guide conduit. Salubria, a biostable polyvinyl alcohol hydrogel manufactured by the Salumedica, mimics the ECM of human tissue due to the hydrogel component, whereas Polyvinyl alcohol (PVA) adds structural stability (Ruiter et al. 2009). However, Salubria nerve guide tubes have been reported to cause compression on the regenerative axonal sprouts at the suture line (Kehoe et al. 2012).

There are several approved NGCs on the market which are made from polyesters. Neurotube®, developed by Synovis, is an NGC manufactured from woven poly(glycolic acid) (Daly et al. 2013). Neurotube® has had reported success bridging injury gaps from 8 to 30 mm, and results are comparable with autograft use for 20 mm gaps (Kehoe et al. 2012). However, reports indicate that Neurotube® has a relatively fast degradation rate, completely degrading by six months, therefore changing the mechanical properties and losing structural integrity before complete reinnervation is established (Kehoe et al. 2012). Poly(glycolic acid) is known to degrade into acidic by-products (glycolic acid) and can result in a localized drop in pH (Duffy et al. 2019). Neurolac™ is manufactured from poly(D,L-lactide-co-ε-caprolactone) by Polyganics. It is a hollow tube and has been reported to bridge nerve injury gaps of up to 20 mm (Arslantunali et al. 2014). Compared with Neurotube®, it degrades more slowly (typically 24 months), and the resultant degradation products (lactic acid and caprolactone) are less acidic (Daly et al. 2013). Clinical studies using Neurolac™ have however reported disadvantages, including device collapse and neuroma formation due to the poly(d-lactide-co-ε-caprolactone) being rigid and inflexible (Kehoe et al. 2012).

A number of particular criteria must be met in order for an NGC to be deemed successful. The biomaterial used must be biocompatible, nontoxic, should not cause an immune response, should ideally be biodegradable, be porous (to allow nutrient transfer), meet handling and sterilization specifications, provide guidance for the regenerating axons, be flexible, exhibit similar mechanical properties to the native nerve, and allow ease of implantation without adding tension to the damaged nerve (Kehoe et al. 2012). Therefore, the material used, the nerve guide design, and the manufacturing method must be taken into consideration beforehand (Bell and Haycock 2012). Although the FDA approved NGCs and devices above have reported success, there are also limitations with using each. Natural materials, though biocompatible and exhibiting mechanical properties similar to native nerve, have issues with batch to batch variability, unwanted immune responses, fast degradation rates, and difficulties in processing (Kehoe et al. 2012). Synthetic materials in contrast can be processed more easily into the required design and have controllable degradation rates. However, issues arise with acidic

degradation products, and typically such materials have stiffer mechanical properties (Nectow et al. 2012). This chapter therefore critiques the most commonly used biomaterials made from natural or synthetic materials for peripheral nerve repair.

3 Natural Materials

Natural materials such as collagen type I and chitosan are currently being used to manufacture FDA approved NGCs (Bell and Haycock 2012). Natural materials offer many advantages as biomaterials for peripheral nerve repair. They are highly biocompatible and promote cellular adhesion by presenting specific biological cues on the material surface to increase protein binding, and in so doing, can control cellular behavior (Magaz et al. 2018). As mentioned above, natural materials exhibit mechanical properties which are similar to native nerve, but limitations arise with their use such as fast degradation rate, unwanted immune response, and batch to batch variability (Nectow et al. 2012). Therefore, investigations into improving natural material conduits, type I collagen and chitosan tubes, are being conducted, as well as investigating new natural materials such as silk, alginate, and hyaluronic acid (Fig. 2) (Arslantunali et al. 2014).

3.1 Collagen

Collagen is the most abundant protein in the human body and is also the most commonly used natural biomaterial for peripheral nerve repair. To date, it has been used to manufacture seven different devices consisting of NGCs and wraps (Bell and Haycock 2012). These devices are mainly fabricated from type I and type III collagen, sourced from bovine and porcine sources (Luca et al. 2014). These are known to have a high degree of purity when extracted, in particular from bovine tendon, as type I collagen. Uncrosslinked collagen has a reported tensile modulus of 16.7 kPa. By crosslinking, tensile modulus increases to 22.5–32.6 kPa, and in so doing, the structural integrity of collagen NGCs can be achieved (Wang and Cai 2010). Collagen has been investigated as both the outer tubular material, as well as for forming intraluminal structures. Waitayawinyu et al. compared collagen conduits and PGA conduits implanted into a 10 mm rat sciatic nerve injury defect for 15 weeks (Waitayawinyu et al. 2007). The collagen conduit and the autograft control had significantly higher axonal counts, wet muscle weight, and muscle contraction force when compared to the PGA conduit. This was concluded as being due to the biological and physical properties of type I collagen (Waitayawinyu et al. 2007).

Collagen filaments and fibers can be manufactured into tubular devices for peripheral nerve repair and have showed some promise in spinal cord injuries (Okamoto et al. 2010; Liu et al. 2012). Okamoto et al. implanted fibrous collagen conduits into 30 mm beagle dog peroneal nerve defects in which nerve regeneration was successful, with minimal scar tissue formation or macrophage infiltration, although complete repair of

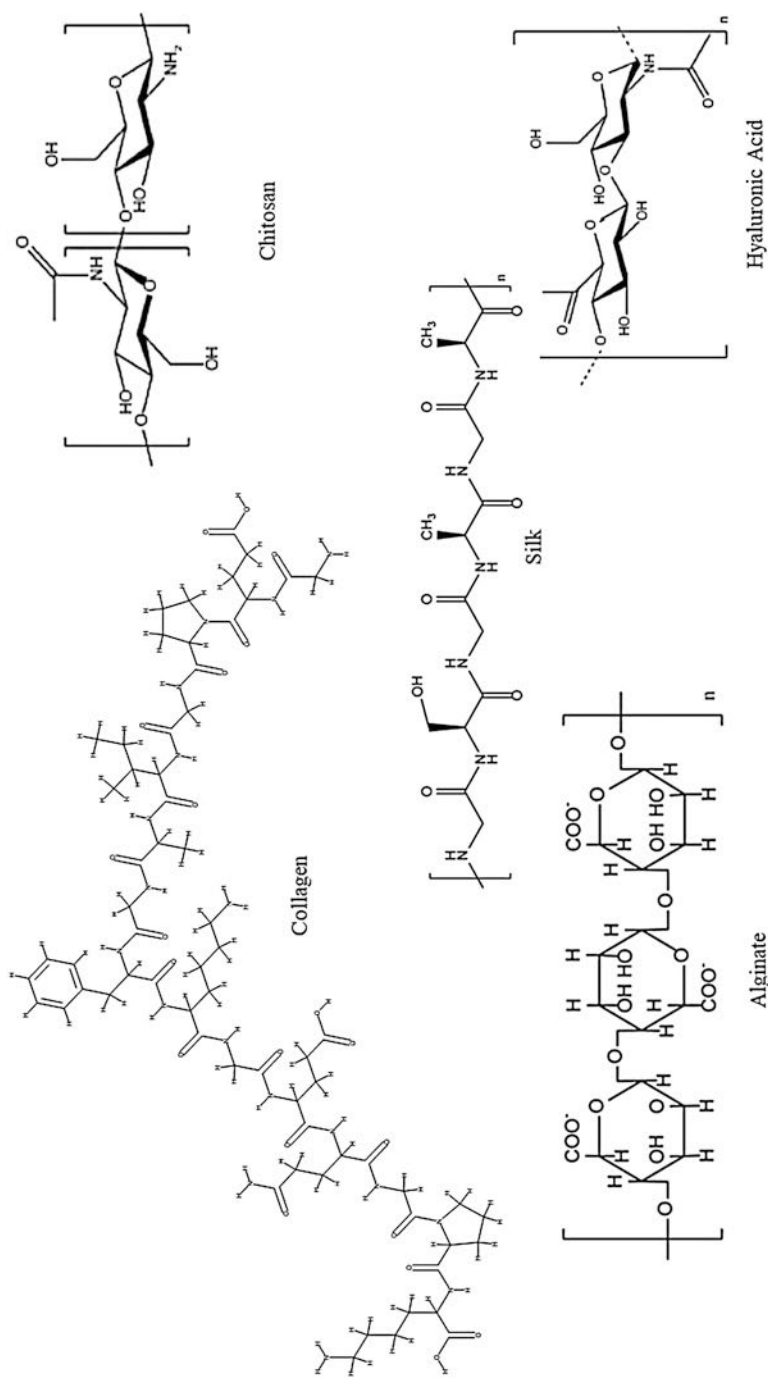


Fig. 2 Chemical structures of collagen, chitosan, silk, alginate, and hyaluronic acid (Structure of collagen reproduced by Chem4Word plugin. Structures of Chitosan and Hyaluronic acid reproduced by Arslantunali et al. (2014) Copyright © 2014 Arslantunali et al. with permission from, and published in Dove Medical Press Ltd. Structures of silk fibroin and alginate reproduced from Shen et al. (2015) Copyright © 2015 Shen et al. with permission from, and published in PLoS ONE)

the peripheral nerve was not achieved over the 52-week time period (Okamoto et al. 2010). Collagen hydrogels have also been investigated for nerve repair, either as hydrogel tubular structures and intraluminal structures (Wang and Cai 2010). Phillips et al. manufactured hydrogel collagen structures containing primary Schwann cells and implanted tubes into a 5 mm rat sciatic nerve injury defect (Phillips et al. 2005). Schwann cell seeded collagen hydrogel tubes were compared with hollow silicone tubes and an empty defect. Collagen hydrogel tubes significantly improved nerve regeneration, with regards to axonal growth and the presence of Schwann cells at the injury site (Phillips et al. 2005). The above studies confirm collagen, as a biomaterial in nerve repair, is effective at promoting nerve regeneration *in vitro* and *in vivo*. However, issues still arise with the use of collagen due to maintaining its structural integrity in long gap injuries and for long term *in vivo* studies (Bell and Haycock 2012). Investigating cross-linkers for collagen, using collagen as an intraluminal filler, blending collagen with synthetic materials, and incorporating growth factors and support cells into collagen hydrogels are all currently being considered as approaches for improving collagen nerve guide conduits (Luca et al. 2014; Wang and Cai 2010; Verdú et al. 2002; Yu et al. 2011).

3.2 Chitosan

The use of chitosan in peripheral nerve repair has gained increased attention and is the material used to manufacture the Reaxon[®] Nerve Guide (Bell and Haycock 2012). Chitin is a naturally occurring polysaccharide derived from the shells and exoskeletons of crustaceans and arthropods and is the second most abundant polysaccharide found in nature, after cellulose (Bak et al. 2017). Chitosan is formed from the N-deacetylation of chitin and exhibits excellent biocompatibility, antimicrobial properties, and mechanical properties that approximate nerve (Arslantunali et al. 2014). Chitosan has a glass transition temperature of approximately 203 °C, and its mechanical properties can vary (Wang and Cai 2010). Chitosan is known to degrade via thermal and enzymatic degradation, in which lysozyme degrades chitosan into small oligosaccharides and glycoaminoproteins, forming by-products that can be metabolized *in vivo* and that are nontoxic (Rodríguez-Vázquez et al. 2015). Chitoooligosaccharide (COS), a degradation product of chitosan, has been shown to have neuroprotective effects and promote Schwann cell proliferation (Boecker et al. 2019).

Chitosan is usually processed in solution, due to its low thermal stability, and can be formed into films, tubular structures, fibers, and hydrogels (Wang and Cai 2010). Wrobel et al. manufactured chitosan films by solvent casting, using ammonia in a methanol/water mixture and sterilization thereafter by electron beam (Wrobel et al. 2014). The Young's modulus of films decreased from 0.47 MPa to 0.41 MPa when using electron beam sterilization. Analysis of chitosan film biocompatibility used rat neonatal Schwann cells, rat adult Schwann cells, immortalized rat Schwann cells, bone marrow derived stem cells, and dissociated rat dorsal root ganglia (DRG) cultures (Wrobel et al. 2014). Overall, chitosan films supported cell

metabolic activity, proliferation, and adhesion, plus neurite outgrowth from primary neurons of dissociated DRG cultures (Wrobel et al. 2014). Chitosan films were then incorporated into the lumen of chitosan nerve guides in the study by Meyer et al. (Meyer et al. 2016). Chitosan nerve guides were manufactured by the extrusion of chitin, with washing and hydrolysis, and chitosan films inserted into NGC lumens in a “Z shape.” Chitosan tubes containing chitosan films enhanced and significantly improved nerve regeneration in a 15 mm rat sciatic nerve defect, with regard to axonal regeneration and functional recovery when compared to hollow chitosan tubes alone (Meyer et al. 2016).

Chitosan can also be electrospun to form fibrous tubular constructs for nerve repair. Wang et al. fabricated randomized and oriented chitosan nanofiber tubular constructs and implanted devices into a 10 mm rat sciatic nerve defect (Wang et al. 2009). Orientated fiber chitosan tubes had a significantly higher tensile strength compared to randomized fiber chitosan tubes, and orientated fiber chitosan tubes enhanced nerve regeneration, and functional recovery compared to randomized fiber chitosan tubes. However, results were not comparable to autograft controls (Wang et al. 2009). Chitosan can also be added to, and blended with, synthetic materials, to improve biocompatibility and mechanical properties (Boecker et al. 2019). It can be processed into hydrogels and used as an outer tube material as well as an intraluminal component material. Freier et al. manufactured chitosan and chitin hydrogel films and tubes by acylation chemistry and mold casting (Freier et al. 2005). Both chitosan and chitin films supported cell adhesion and differentiation in which neurite outgrowth from DRG explants was significantly longer compared to chitin films (Freier et al. 2005). Chitosan can also be processed into microspheres, sponges, and porous conduits, and as such has been investigated for peripheral nerve repair applications (Boecker et al. 2019).

3.3 Silk Fibroin

The use of silk has gained attention recently as a biomaterial for nerve repair as it is inexpensive, and has ideal mechanical and biological properties (Magaz et al. 2018). Silk is a naturally occurring fibrous protein and is produced from spiders and silkworms (Holland et al. 2019). Silkworm silk fibroin has a Young's modulus of 10–17 GPa and ultimate tensile strength of 300–740 MPa (Koh et al. 2015). Silk is known to degrade in vivo by enzymatic degradation and its degradation rate can be tuned from months to years depending on the material processing technique (Holland et al. 2019). Silk can withstand temperatures of up to 250 °C and can be processed into tubular structures, fibers, and hydrogels for peripheral nerve repair (Magaz et al. 2018). Huang et al. cast *Bombyx mori* fibroin into tubular structures, with Spidrex[®] fibers in the lumen of the tube, for implantation into a 10 mm rat sciatic nerve defect (Huang et al. 2012). Silk conduits, containing Spidrex[®] fibers, supported excellent nerve regeneration, with similar results to the autograft controls with regards to functional recovery, inflammatory response, and Schwann cell support (Huang et al. 2012). Ebrahimi et al. manufactured nanofibrous

silk conduits, aligned and randomly orientated fibers by electrospinning, and implanted tubes, with and without Schwann cells, into a 10 mm rat sciatic nerve defect (Ebrahimi et al. 2018). At 4 months' implantation, the aligned silk fiber tube, containing Schwann cells, had the thickest myelin sheath and the largest diameter of myelinated fibers, similar to the autograft control (Ebrahimi et al. 2018). Yang et al. used a casting method to manufacture silk conduits and filled the lumen of the tubes with aligned silk fibers. Conduits were implanted into a 10 mm rat sciatic nerve injury defect. After 6 months, silk conduits promoted nerve regeneration across the defect gap, in which thickness of the myelin sheath and diameter of myelinated nerve fibers were similar to the autograft controls (Yang et al. 2007). Silk has also been fabricated into hydrogels and blended with other materials, such as synthetic materials, for use in nerve repair (Li et al. 2012). These studies highlight the potential of using silk in peripheral nerve repair.

3.4 Alginate

Alginate is a naturally occurring polysaccharide that can be extracted from brown seaweed, such as *Laminaria hyperborean* and *Laminaria digitate*, and can also be produced by bacterial fermentation using *Azotobacter* and *Pseudomonas* (Lee and Mooney 2012). It is low-cost to produce, biocompatible, has chelating abilities, and is used in regenerative medicine applications (Boni et al. 2018). Alginate is nondegradable naturally, but degradable forms of alginate can be produced by physical and chemical crosslinking, and so degradation rate and therefore mechanical properties of cross-linked alginate can be tailored according to the application (Sun and Tan 2013). Mechanical and physical properties of cross-linked alginate forms can also be tailored by the molecular weight of the alginate, and viscosity of the solution formed (Boni et al. 2018). The molecular weight of commercial alginate varies from 32,000 to 400,000 g/mol, and by mixing low and high molecular weight alginates together, viscosity and stiffness of alginate gels can be controlled (Lee and Mooney 2012).

Alginate is commonly processed into hydrogels for tissue engineering applications, forming sponges/porous structures and fibers for peripheral nerve repair (Lee and Mooney 2012). Suzuki et al. manufactured porous freeze-dried alginate tubes, covered by a PLGA mesh, and implanted devices into a 50 mm gap sciatic nerve defect in the cat (Suzuki et al. 1999). Regeneration of the nerve occurred over the injury gap as seen by the presence of new nerve fascicles. Nerve function occurred at 13 weeks' implantation, and there was little evidence of inflammation, suggesting that the alginate gel enhanced nerve regeneration, although this was not compared to an autograft control (Suzuki et al. 1999). The same group moved away from tubular structures and implanted freeze dried alginate sponges into 10 mm rat sciatic nerve defects (Hashimoto et al. 2002). Hashimoto et al. reported that after 4 weeks' implantation, axon regeneration and Schwann cell migration was observed at the distal stump using alginate gel sponges. After 12 months' implantation, alginate gels

were favorable over collagen gel and fibrin glue, though these were not comparable with the autograft control (Hashimoto et al. 2002).

Alginate hydrogels can also be used as intraluminal fillers for peripheral nerve repair. Mosahebi et al. filled Poly-3-hydroxybutyrate (P(3HB)) conduits with alginate hydrogels, containing Schwann cells and fibronectin (Mosahebi et al. 2003). Conduits were implanted into a 1 cm rat sciatic nerve defect and implanted for 2, 3, and 6 weeks before assessment for axonal regeneration and Schwann cell viability (Mosahebi et al. 2003). Overall, the addition of alginate/fibronectin gels plus Schwann cells significantly enhanced axonal regeneration compared to empty conduits alone (Mosahebi et al. 2003). Although inert, alginate gel alone lacks bioactive ligands to aid cellular adhesion, the addition of fibronectin to the gel-supported axonal regeneration, and Schwann cell adhesion was greatly improved (Sun and Tan 2013; Mosahebi et al. 2003). Alginate can be fabricated into fibers for peripheral nerve repair. Szarek et al. manufactured polyurethane (PU) and polylactide (poly L-lactide-co-D,L-lactide) (PLDL) conduits by dip coating, and incorporated alginate fibers (sourced from the Department of Artificial Fibres, Lodz University of Technology, Poland) into the lumen of the conduits (Szarek et al. 2013). The chemistries investigated were comprised of calcium alginate fibers, sodium alginate fibers, and a 50/50 mixture. Fiber-filled conduits were implanted into a 10 mm rat sciatic nerve defect for 12 weeks (Szarek et al. 2013). Overall, calcium alginate fibers supported nerve regeneration more effectively than empty tubes, sodium alginate fiber-filled tubes, or a 50/50 mixture of filled tubes. However, the results were not comparable to control or autograft (Szarek et al. 2013). Alginate can also be 3D printed and formed into microspheres for nerve repair (Sun and Tan 2013). The use of alginate in peripheral nerve repair certainly has potential, but typically requires blending with synthetic or natural materials as above, in order to impart function, initially as cellular adhesion (e.g., Schwann cells) and thereafter for nerve regeneration potential (Lee and Mooney 2012).

3.5 Hyaluronic Acid

Hyaluronic acid (HA) is a naturally occurring glycosaminoglycan found in the extracellular matrix (Wang and Cai 2010). In the body, HA is a major component of connective tissue, found in most organs, and has an important role in wound healing, reducing scar formation (Wang et al. 2012). It is a polymer of repeating disaccharide units, and its molecular weight can vary from 10 to 1000 kDa (Vasvani et al. 2019). Due to the variety in molecular weight, HA can be processed into a variety of forms and is an advantageous material for regenerative medicine. It has viscoelastic properties, is biocompatible, biodegradable, hydrophilic in nature, and is resorbable in the body (Boni et al. 2018). HA degrades via enzymatic degradation *in vivo*, targeted by a family of hyaluronidases, and degraded into smaller oligosaccharides that are readily removed by the body (Vasvani et al. 2019). HA can be processed into a variety of forms for nerve repair applications such as hydrogels and fibers (Arslantunali et al. 2014). Sakai et al. fabricated cross-linked HA conduits by

UV curing the gel in a mold and filled conduits with a collagen hydrogel containing Schwann cells, plus neurospheres derived from rat hippocampus (Sakai et al. 2007). HA tubes were cultured in vitro and after 3 weeks HA conduits remained intact, maintaining size and architecture. Viable Schwann cells and neurospheres could be visualized, as well as neurite outgrowth from the neurospheres (Sakai et al. 2007).

HA hydrogels can be used as an intraluminal scaffold for nerve guide conduits. Li et al. used empty chitosan conduits, chitosan conduits plus an internal HA hydrogel and HA hydrogels at a clamp injury site to the sciatic nerve (Li et al. 2018). No significant differences were detected in the groups with regard to scarring – and the addition of HA gel to chitosan conduits promoted nerve regeneration with an “increased number of axons, nerve fiber diameter and myelin thickness” compared to empty chitosan conduits and HA gel alone (Li et al. 2018). HA can also be processed into fibers for nerve repair. Jansen et al. fabricated Hyaff-11 (an esterified form of hyaluronan) into tubes by weaving individual Hyaff strands and coating with a thin layer of Hyaff-11 polymer (Jansen et al. 2004). Cellular responses were investigated in vitro and degradation of conduits investigated in vivo. Hyaff-11 conduits were not cytotoxic and were implanted into subcutaneous pockets on the back of a rat. A mild immune reaction was observed after implanting Hyaff-11 tubes, and degradation was observed after 3 weeks’ implantation (Jansen et al. 2004). In analogous work, Liou et al. fabricated a nanofibrous gelatin together with a hyaluronan-gelatin gel by electrospinning and investigated RT4-D6P2T rat Schwann cell responses in vitro (Liou et al. 2013). Cell attachment and proliferation was similar on both gelatin and HA-gelatin fibers, however, Schwann cells expressed higher levels of Nrg1, GFAP, P0 mRNA, Nrg1, and P0 proteins when grown on HA-gelatin fibers, compared to gelatin alone (Liou et al. 2013). Hyaluronic acid is a versatile material in regard to physical processing and can take many forms including viscoelastic solutions, hydrogels, electrospun fibers, nonwoven meshes, macroporous and fibrillar sponges, flexible sheets, and nanoparticulates (Arslantunali et al. 2014). Thus, HA has emerging value when considering the biochemical properties for supporting regeneration, in combination with versatility to structure into different physical forms for scaffold and device utility.

Overall, natural materials offer many advantages for peripheral nerve repair. A common and emerging theme is the combination of natural materials that are blended with a wide range of other natural materials to improve structural integrity of scaffold devices. Other natural materials of interest include glycosaminoglycans, laminin, fibronectin, fibrin, gelatin, and keratin (Arslantunali et al. 2014).

4 Synthetic Materials

Biodegradable synthetic polymers are also commonly used for peripheral nerve repair. A key advantage of synthetic biomaterial use is an ability to readily control physical and chemical properties, such as mechanical properties, surface chemistry, and surface topography to improve cellular adhesion, proliferation, and differentiation (Bell and Haycock 2012). Synthetic polymers can be processed by a number of

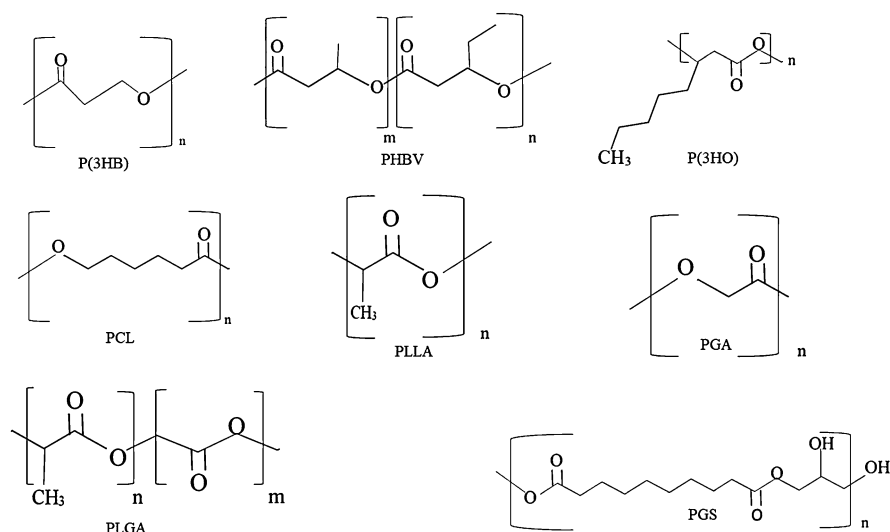


Fig. 3 Chemical structures of P(3HB), PHBV, P(3HO), PCL, PLLA, PGA, PLGA, and PGS

manufacturing methods, such as solvent casting, 3D printing, and melt extrusion (Duffy et al. 2019). Therefore, both simple and complex conduit designs can be easily manufactured. Synthetic materials used in peripheral nerve repair include polyhydroxyalkanoates, polycaprolactone, poly(L-lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid), and poly(glycerol sebacate) (Fig. 3).

4.1 Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates (PHAs) are a family of intracellular biopolymers, produced by both gram negative and gram positive bacteria under conditions when growth nutrients are limited (Valappil et al. 2006). It is debatable whether these materials are classified as natural or synthetic, given the natural origin of their synthesis versus the ability to precisely control properties such as molecular weight, and thereafter a wide range of physicochemical properties. However, for the purposes of this review, we shall include them in Sect. 4, with this caveat. PHAs are biocompatible, biodegradable, and are classified into short chain length (SCL) and medium chain length (MCL) PHAs, depending on the number of carbon atoms in their monomer unit (Basnett et al. 2013). Short chain PHA monomers consist of 3–5 carbon atoms, whereas medium chain PHAs consist of 6–14 carbon atoms (Philip et al. 2007). Physical properties SCL-PHAs and MCL-PHAs vary due to the differences in carbon atoms (Lizarraga-Valderrama et al. 2016). SCL-PHAs, such as poly(3-hydroxybutyrate) (P3HB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), experience high melting temperatures, high crystallinity, higher melting, and are more brittle (Bağ et al. 2017). MCL-PHAs, such as polyhydroxyoctanoate

(P3HO), have low melting temperatures, low crystallinity, and are very elastomeric due to long aliphatic chains in their structure (Basnett et al. 2013). PHAs degrade by hydrolytic degradation (surface erosion), via de-esterification, and the by-products of degradation, such as D,L-hydroxybutyrate, are removed by metabolic pathways in the body (Assaf et al. 2017). Due to their natural origin, PHAs are an attractive polymer in nerve tissue engineering, as they offer a range of different mechanical and physical properties, are biocompatible, and biodegradable, and P(4HB) (poly(4-hydroxybutyrate)) has FDA approval as a suture material (Waitayawinyu et al. 2007). PHAs currently investigated in peripheral nerve repair include P(3HB), PHBV, and P(3HO) (Arslantunali et al. 2014).

P(3HB) was one of the first PHA materials investigated for peripheral nerve repair. Hazari et al. reported successful bridging of a 1 cm rat sciatic nerve defect, although results were not comparable to autograft use, identified as a lack of cellular events occurring in the P(3HB) conduit (Hazari et al. 1999). Young et al. reported bridged gaps of up to 40 mm in rabbits using P(3HB) conduits, though they identified that a hollow tube was not sufficient for optimal nerve regeneration and repair (Young et al. 2002). Mosahebi et al. reported bridged injury gaps, up to 10 mm, in rat sciatic nerve using P(3HB) conduits filled with an alginate/fibronectin hydrogel (Mosahebi et al. 2003). As a highly brittle material, P(3HB) has been blended with other materials, other PHAs and natural materials, to improve its mechanical properties, as well as formed into copolymers (Philip et al. 2007). An analogous material, PHBV, is a copolymer of P(3HB) and the incorporation of a 3-hydroxyvalerate monomer. This decreases the glass transition temperature, decreases polymer brittleness, and can be more readily processed (Zhao et al. 2003). Karimi et al. (2014) reported successful nerve regeneration, bridging 30 mm rat sciatic nerve gaps, using a porous oxygen plasma-treated PHBV nerve guide conduit with no significant differences detected in the thickness of the reformed myelin sheath, or motor recovery, compared to autograft use (Biazar and Keshel 2013). Other copolymers of SCL-PHAs have been investigated for peripheral nerve repair including poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB) and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)(PHBHHx) (Xu et al. 2010).

P(3HO) has been investigated for regenerative medicine applications, predominantly due to its elastomeric properties. However, there are very few published studies on P(3HO) for peripheral nerve repair. Hazer et al. reported a good tissue response, when implanting solvent casted P(3HO) tubes into a 10 mm rat sciatic nerve injury gap (Hazer et al. 2013). However, electrophysiological and immunohistochemical studies identified less neuroregeneration compared to autograft use (Hazer et al. 2013). There are a few published studies in which P(3HO) has been blended with other PHAs, such as P(3HB), to improve the mechanical properties of the more brittle PHAs (Basnett et al. 2013). Lizarraga-Valderrama et al. manufactured blends of P(3HO)/P(3HB) with ratios of 75:25, 50:50, and 25:75, in which the addition of P(3HO) to P(3HB) improved mechanical properties, and neuronal cell attachment, differentiation, and neurite outgrowth was significantly superior on blends containing higher amounts of P(3HB) (Lizarraga-Valderrama et al. 2015). As a result, aligned micrometer fibers of the 25:75 P(3HO)/P(3HB)

blend were manufactured, with varying diameters as an intraluminal guidance structure (Lizarraga-Valderrama et al. 2019). Copolymers of P(3HO), such as P(3HO-co-3HD) and P(3HO-co-3HD-co-3HDD), are currently being investigated.

4.2 Polycaprolactone (PCL)

Polycaprolactone (PCL) is a semicrystalline, aliphatic linear polymer and is synthesized by the polymerization of cyclic monomer ϵ -caprolactone (Duffy et al. 2019). PCL has a glass transition temperature of -54°C and melting temperature between 55°C and 60°C (Ulery et al. 2011). It is a highly hydrophobic polymer, due to long aliphatic chains in the structure. It is elastomeric, with a low tensile strength of around 23 MPa and a high elongation at break property (470%) (Ulery et al. 2011). PCL degrades slowly via hydrolytic degradation due to polymer backbone stability (Ulery et al. 2011). Succinic acid, valeric acid, and butyric acid are the products of PCL degradation and are not toxic to the body (Kehoe et al. 2012; Reid et al. 2013). Enzymatic degradation of PCL can also occur slowly, however, the slow degradation rate can be a useful quality for regenerative medicine applications (Ulery et al. 2011). Poly D,L lactide-co-caprolactone (a copolymer of PCL), is used to manufacture the NGC Neurolac[®] (Kehoe et al. 2012). Success is reported using Neurolac[®] conduits, which can bridge nerve injury gaps of 20 mm. They have a slow degradation rate, but also conduit inflexibility and high rigidity that can cause neuroma formation (Bell and Haycock 2012). Therefore, emerging studies using PCL as a material for nerve repair investigate changing the conduit design, improving the mechanical properties, as well as tailoring the degradation rate.

Assaf et al. incorporated carbon and graphene nanoparticles into solvent casted PCL conduits (Assaf et al. 2017). Using PCL, decreased the risk of tube encapsulation, and using both carbon and graphene nanoparticles increased the PCL conduit degradation rate. The number of regenerated axons in PCL tubes with both carbon and graphene nanoparticles increased, compared to PCL conduits alone when using a 4 mm rat sciatic nerve injury model (Assaf et al. 2017). Sun et al. reported increased NG108-15 neuronal cell and primary Schwann cell adhesion on sodium hydroxide-treated PCL films compared to nontreated PCL films (Sun et al. 2009). Solvent casted films were rolled into circular conduits for in vivo testing. After 2 weeks' implantation, early nerve regeneration was confirmed in a 10 mm rat sciatic nerve injury model using the sodium hydroxide-treated PCL conduits (Sun et al. 2009). After 18 weeks' implantation, Reid et al. reported similar numbers of regenerated axons, and myelinated axons, in both the proximal and distal nerve stumps using both sodium hydroxide treated PCL conduits and the autograft control (Reid et al. 2013). After 18 weeks' implantation, conduits remained intact, and reinnervation of end organ muscle and skin was evident, although end muscle weight in the autograft was significantly heavier, highlighting the need for further studies to improve bioengineered nerve guide conduits (Reid et al. 2013). In summary, these

studies highlight that hydrophobic PCL can be modified to increase hydrophilicity, increasing cellular adhesion, and that the degradation rate, of PCL, can be increased (Sun et al. 2009).

4.3 Poly(L-lactic acid) (PLLA)

Poly(L-lactic acid) (PLLA) is another polyester of interest for peripheral nerve repair (Arslantunali et al. 2014). PLLA is synthesized by the polymerization of L-lactide and is a semicrystalline aliphatic polyester (Tokiwa and Calabia 2007). PLLA has a melting temperature of 175 °C, a glass transition temperature of 60–65 °C, and a Young's Modulus of 4.8 GPa (Rodríguez-Vázquez et al. 2015). PLLA is biocompatible, hydrophobic in nature, and degrades via hydrolytic bulk erosion into acidic degradation products, such as lactic acid (Casalini et al. 2019). As degradation is slow, due to random ester bond cleavage, PLLA is often blended with other synthetic polyesters to increase degradation rates (Elsawy et al. 2017). PLLA has been investigated for use in peripheral nerve repair to manufacture nerve guide conduits. Evans et al. manufactured porous PLLA conduits from solvent casting, extrusion, and salt porogen leaching and implanted conduits into a 10 mm rat sciatic nerve injury model (Evans et al. 1999). After 16 weeks in the distal sciatic nerve, nerve fiber density in PLLA conduits was comparable to the autograft control, and axon number and nerve area were also comparable (Evans et al. 1999). Recently, Frost et al. manufactured PLLA nerve guide conduits from electro-spinning (Frost et al. 2018). Compared to PCL electrospun conduits, electrospun PLLA nerve guides maintained structural integrity, had better mechanical properties, and supported *in vivo* axonal regeneration (Frost et al. 2018). However, both PLLA and PCL electrospun conduits were inserted into silicon tubes before implantation to maintain structural integrity, but the results of the study highlighted the use of PLLA electrospun conduits as a carrier for cellular components, such as autologous Schwann cells and stem cells (Frost et al. 2018). PLLA is a less favorable polyester for nerve repair, but it is still highly researched due to its physical and mechanical properties (Arslantunali et al. 2014).

4.4 Polyglycolic Acid (PGA)

Poly(glycolic acid) is a biodegradable polyester used commonly for several medical applications including sutures. It has also been used to construct nerve guides. PGA is highly crystalline, with thermoplastic and aliphatic properties (Arslantunali et al. 2014). It has a melting temperature of 224 °C, a glass transition temperature of 35–40 °C, and a Young's modulus of 12.5 kPa (Kehoe et al. 2012). PGA degrades via hydrolytic bulk erosion and is reported to degrade within 6–12 months (Duffy et al. 2019). Degradation products of glycolic acid, glycolate, pyruvate, and carbon dioxide are acidic. In comparison with PLLA, its use in

biomedical engineering can be hindered in some applications by an inappropriately fast degradation rate and the local generation of acidic products (Kehoe et al. 2012). Compared to PLA, it is more hydrophilic in nature (Gentile et al. 2014). PGA is the main component of the nerve guide conduit, Neurotube[®], which gained FDA approval in 1999 and is still investigated in peripheral nerve repair (Bell and Haycock 2012). The study by Dellon et al. was one of the first to report on the use of PGA conduits as an alternative to autografts, after positive results were observed in a 3 cm primate ulnar nerve injury model (Dellon and Mackinnon 1988).

Weber et al. reported on the first randomized study of PGA for digital nerve repair in humans. The criterion for the study was complete division of a sensory nerve with a deficit of 3 cm or less (Weber et al. 2000). Improved sensation was reported using a PGA conduit compared to end-to-end suturing with injury gaps less than 4 mm. Equivalent results were observed using both PGA conduits and autograft use for injury gaps up to 3 cm (Weber et al. 2000). Rosson et al. investigated PGA conduits for short-gap repair (1.4–4 cm gaps) in motor nerves, in which all patients regained some sensation and functionality (Rosson et al. 2009).

4.5 Poly Lactic-co-Glycolic Acid (PLGA)

PLGA is a copolymer of PLA and PGA, formed via random copolymerization, and investigated for use in peripheral nerve repair (Ulery et al. 2011). PLGA is an advantageous material as mechanical properties, physical properties, and degradation rate can be tailored, by varying the amounts of both monomers (Arslantunali et al. 2014). Higher amounts of lactic acid in the copolymer result in a more hydrophobic polymer and a slower degradation rate (Gentile et al. 2014). Therefore, the Young's modulus ranges 1–4 GPa, the glass transition temperature 40–55 °C, and the melting temperature 75–200 °C (Duffy et al. 2019). PLGA degrades via hydrolysis, through bulk erosion, into its monomer components of lactic and glycolic acid (Gentile et al. 2014). PLGA first received FDA approval in 1974 for use as the suture material Vicryl[®] (Ethicon), and more recently Vicryl Rapide (Ulery et al. 2011). As a result, PLGA is being researched extensively for nerve tissue engineering applications. Oh et al. manufactured PLGA tubes from immersion precipitation, by mixing PLGA with polyol F127, a surfactant, in which inner pores of 50 nm and outer pores of 50 µm were also created (Oh et al. 2008). After implantation for 24 weeks, PLGA-F127 tubes supported significantly larger diameters of myelinated axons, and thicker myelin sheaths, compared to silicone control tubes in a 10 mm rat sciatic nerve injury model (Oh et al. 2008). Shen et al. implanted PLGA conduits, manufactured by weaving, treated with oxygen plasma, and coated with chitosan and ciliary neurotrophic factor (CNTF) into canine tibial defects of 25 mm, for 3 months (Shen et al. 2010). Overall, PLGA-chitosan conduits plus CNTF were comparable with an autograft control, and the addition of the CNTF increased nerve tissue percent, average nerve fiber diameter, and the thickness of the myelin sheath, compared to the PLGA/chitosan conduits alone (Shen et al. 2010).

4.6 Poly(glycerol sebacate) (PGS)

Poly(glycerol sebacate) PGS is a biodegradable elastomeric polyester, primarily investigated for soft tissue engineering applications (Loh et al. 2015). It is synthesized by the polycondensation reaction of glycerol and sebacic acid and is nontoxic and inexpensive to manufacture (Ghafaralahi et al. 2018). PGS has a Young's modulus in the range of 0.01–1.5 MPa, and its mechanical properties can be tailored by changing the curing temperature (Loh et al. 2015). PGS has a melting temperature between 5 °C and 38 °C, and a glass transition temperature between –37 °C and –80 °C (Rai et al. 2012). It is a hydrophilic material that degrades by surface erosion into nontoxic by-products of glycerol and sebacic acid that are metabolized by the body (Denis et al. 2019). PGS exhibits advantageous properties for use in peripheral nerve repair. Sundback et al. investigated PGS for nerve repair in 2005. Compared to PLGA (50:50) films, PGS films supported Schwann cell adhesion and proliferation. Sheets were then implanted underneath the sciatic nerve, on the muscle bed, to observe tissue response (Sundback et al. 2005). A minimal inflammatory response was observed using PGS compared to PLGA. This was attributed to the difference in degradation mechanism, with PGS being gradually absorbed in contrast to PLGA, which experiences a late rapid bulk degradation (Sundback et al. 2005). Singh et al. synthesized a novel photocurable form of PGS, PGS methacrylate (PGSm), to fabricate PGSm hollow nerve guides using additive manufacture technique microstereolithography (Singh et al. 2018). Physical properties of PGSm could be tailored by the degree of methacrylation, such as Young's modulus, water contact angle, and enzymatic degradation. In vitro analysis of PGSm films with varying degrees of methacrylation supported neuronal and Schwann cell attachment, and neuronal cell neurite outgrowth (Singh et al. 2018). In vivo, PGSm conduits directed axonal growth and supported regeneration of axons, however, the autograft control was superior (Singh et al. 2018). These studies highlight PGS as promising material in peripheral nerve repair.

5 Improving Existing Hollow Nerve Guide Conduits

NGC designs must balance effective treatment for supporting nerve reinnervation with safety, under the governance of national and international medical device legislation. Improvements to novel biomedical materials are of great importance worldwide. It is widely recognized that a rapidly growing and ageing population places demands on the majority of developed and developing healthcare systems across the world, with demand for more effective but also cost-effective healthcare interventions. The medical technology sectors in the UK, USA, EU, and Australasia have doubled since 2009. Biomaterials are key components of many implantable devices including NGCs but more commonly exist in areas of orthopedic implants (artificial hips, knees, shoulders, and bone grafts), cardiovascular implants (heart valves, pacemakers, catheters, grafts, and stents), and dental implants (enamel,

fillings, prosthetics, and orthodontics). This growth in the biomedical materials sector is being driven by increasing healthcare needs of the geriatric population, advancements in medical technologies, and rising incidences of chronic diseases, such as osteoarthritis, cardiovascular diseases, neuropathic diseases, and congenital disorders. The relevance of this to nerve injury repair is the emergence of biomaterials that address the current limitations of clinical effectiveness. NGC designs using “advanced” biomaterials are beginning to emerge that promote tissue integration and repair through the development of novel chemistries, physical properties, surface chemistries, and manufacturing and structural designs, in combination with biologics to actively stimulate repair.

Current FDA approved nerve guide conduits are simple hollow tubes, manufactured by both natural and synthetic materials (Bell and Haycock 2012). Several approaches to improve current NGCs exist, such as changing the material of the NGC, changing the design of the conduit, the addition of intraluminal guidance scaffolds, the use of nerve guide coatings, surface modifications, the use of growth factors, and the addition of supporting cells, autologous Schwann cells, and stem cells (as summarized in Fig. 4) (Daly et al. 2012). Many of these approaches are still in the investigation phase but show promise, e.g., Neuromaix[®] nerve guide, which incorporates an intraluminal collagen type I sponge scaffold in the lumen of the tube (Bozkurt et al. 2014). Often a combinational approach is used in studies in which successful nerve reinnervation has been achieved (Shen et al. 2010).

5.1 Changing the Material of the Hollow Tube

One approach to improve nerve guide conduits is to change the outer hollow tube material altogether. Although current NGCs are manufactured from FDA approved polymers, such as collagen and PLGA, there are several disadvantages associated with their use (Nectow et al. 2012). Therefore, using new materials such as silk, alginate, PGS, and PHAs are being investigated (discussed above). Another approach is by blending polymers together to achieve the desired mechanical properties, biocompatibility, and degradation rates optimal for nerve regeneration (Lizarraga-Valderrama et al. 2015). A reasonably common approach is to blend synthetic polymers with natural polymers, such as collagen (Lee et al. 2006). The resulting polymer blend can more readily be processed, using established manufacturing methods, which have a tailored and controlled degradation rate (via the synthetic material component) and have improved biocompatibility and mechanical properties than the natural material component alone (Arslantunali et al. 2014). Collagen is the most commonly used natural material for blending together with synthetic materials and has been blended with PLLA, PCL, PLGA, and PHAs (Baumann and Pham-Dinh 2001). Yu et al. reported improved elastic and hydrophilic properties using PLC-collagen electrospun conduits, when compared to PCL conduits alone (Yu et al. 2011). In vitro results reported electrospun PCL-collagen meshes supported Schwann cell adhesion and proliferation, and in vivo studies showed promising results using collagen-polycaprolactone electrospun

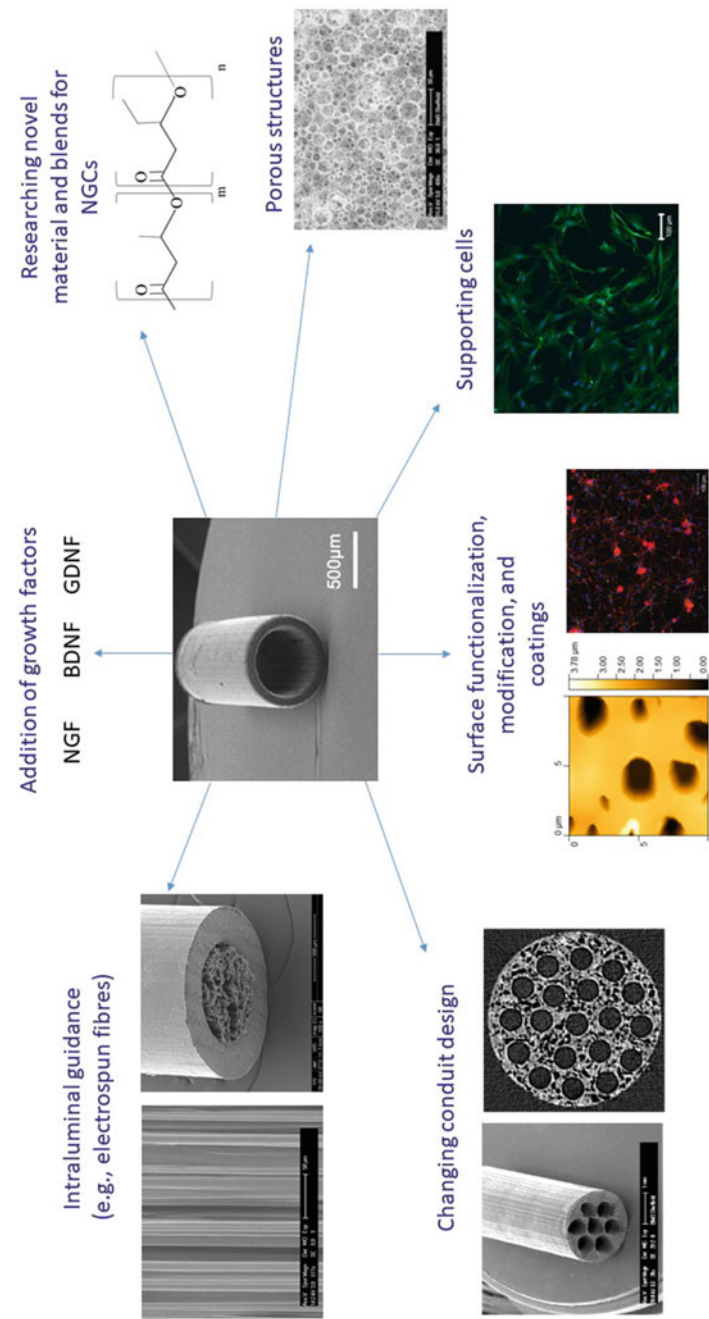


Fig. 4 Experimental approaches to improving existing hollow nerve guide conduits (An NGC containing aligned electrospun fibers for intraluminal guidance, produced by J. Field using the methods described in “Manufacture, characterisation and in vivo assessment of nerve guidance conduits containing physical guidance cues” J. Field (2019) Copyright © J. Field with permission from J. Field. Image of porous structure reproduced from Owen et al. (2016) Copyright © 2015 Owen et al. with permission from, and published in Elsevier Ltd. Image of “changing conduit design” reproduced from Diez-Ahedo et al. (2020) Copyright © 2020 Diez-Ahedo et al. with permission from, and published in Polymers, EISSN 2073-4360, Published by MDPI AG)

conduits, bridging an 8 mm rat sciatic nerve injury gap (Yu et al. 2011). Li et al. fabricated nanofibrous silk-PLGA conduits for in vivo assessment in 5 mm rat sciatic nerve injury defects (Li et al. 2012). Functional recovery of nerves and nerve repair was observed using PLGA-silk fibroin conduits, however, TEM and histological assessment confirmed autograft was still superior (Li et al. 2012). In summary, these studies show promise in the use of blending synthetic materials with biomaterials to manufacture bioactive nerve constructs for peripheral nerve defect repair.

5.2 Changing the Conduit Design

Current NGCs are very simple in design, typically as hollow tubes without grooves, pores, or guidance cues. The addition of channels, microgrooves, and pores can facilitate nutrient transfer and provide guidance for the regenerating axons (Daly et al. 2012). The manufacturing method of the NGC is very important in determining many factors in nerve repair, such as porosity of the conduit and devices with specific details (Bell and Haycock 2012). Methods for manufacturing NGCs include injection molding, mandrel coating, freeze drying, dip-coating, solvent casting, 3D printing, and electrospinning (Pateman et al. 2015; Duffy et al. 2019).

5.3 Addition of Channels and Microchannels

The addition of channels and microchannels is an approach to improve NGCs, providing guidance for the regenerating axons and space for individual nerve fascicles (Lowe and Anderson 2015; Daly et al. 2012). Mechanical and physical properties of collagen conduits, with 1, 4, and 7 channels, were investigated by Yao et al. comparing results with NeuraGen[®] conduits (Yao et al. 2010). Collagen conduits were cross-linked with “0–60mM (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide [EDC] in N-hydroxysuccinimide [NHS])” to improve the swelling and degradation rate (Yao et al. 2010). Overall, decreased swelling, increased degradation, and a lower compressive strength were observed using cross-linked collagen NGCs to NeuraGen[®] conduits. No significant differences were detected between cross-linked collagen films and plain collagen films in rat DRG neurite outgrowth, and the addition of the cross-linkers did not cause any toxicity (Yao et al. 2010).

5.4 Addition of Grooves

It is well reported that surface topography can influence cell attachment and cell differentiation (Chen et al. 2018). Sun et al. reported NaOH treated PCL and PCL-PLA films for nerve regeneration, in which films supported NG108-15 neuronal cell differentiation when rolled into conduits, nerve regeneration across a 10 mm rat

sciatic nerve injury defect (Sun et al. 2009). Sun et al. further improved PCL and PCL-PLA nerve guides by incorporating microgrooves into the films using photolithography in which the width of spacing was 5, 10, 15, and 20 μm , and the width of the grooves were 5, 10, 15, and 20 μm (Sun et al. 2010). NG108-15 neuronal cell and primary Schwann cell alignment were best on grooves that were 20 μm in depth and had width spacing of 5 and 10 μm (Sun et al. 2010). Mobasseri et al. using PCL-PLA films, created NGCs with different grooved films with different patterns, such as “sloped walls (SL), V-shaped (V), and square-shaped (SQ)” (Mobasseri et al. 2015). The depth of the grooves, in all three patterns was 5 μm , and the width of the grooves in the patterns were 15–20 μm (with ridges of 3–6 μm), 15–20 μm (with no ridges), and 10 μm (with ridges of 10 μm), respectively (Mobasseri et al. 2015). SL and V grooved surfaces supported an increase in differentiated adipose derived stem cell attachment compared to SQ grooved and nongrooved surfaces, and nongrooved surfaces. SL and V grooved surfaces were implanted into a 10 mm rat sciatic nerve injury defect for 3 weeks, with SL grooved conduits supporting nerve regeneration significantly better compared to V grooved surfaces (Mobasseri et al. 2015). A 16 week in vivo study indicated no significance between the autograft control and SL grooved conduits with regard to the area of axons, area of myelin, number of axons, muscle wet weight, skin reinnervation, and electrical stimulation of the gastrocnemius muscle (Mobasseri et al. 2015).

5.5 Incorporation of Porosity

Introducing porosity into a nerve guide conduit is an advantageous approach to improving hollow NGCs to allow the transfer of nutrients to and from the injury site, keeping in essential growth factors for nerve regeneration (Kehoe et al. 2012). The addition of pores can decrease mechanical strength of a conduit, and so, factors such as pore size have to be considered carefully in the context of nerve guide design (Bell and Haycock 2012). It has been reported that the ideal pore size for NGCs is between 5 and 30 μm to allow cell proliferation and prevent excessive inflammatory cells penetrating the injury site (Jiang et al. 2010). Pores are incorporated into NGCs by using porogens, immersion precipitation, emulsion templating, and electrospinning. By using gas foaming, Yang et al. manufactured porous PLGA NGCs using salt, controlling the porosity of the of conduits by mixing ratios of the porogen with PLGA (Yang et al. 2005). Mechanical properties could be controlled by varying the porosity of conduits produced, and tissue penetration was enhanced in porous conduits by subcutaneous implantation (Yang et al. 2005). Oh et al. incorporated pores into PCL conduits by immersion precipitation using Pluronic F127. Different ratios of Pluronic F127 created nanopores (~ 100 nm) and micropores (~ 200 μm) in which NGCs with nanopores supported nerve regeneration across a 10 mm rat sciatic nerve injury model, compared to micropored NGCs (Oh et al. 2013). Johnson et al. showed that high internal phase emulsions (HIPEs) could be used to 3D print porous tubular structures using microstereolithography (Johnson et al. 2013). Porous NGCs can also be manufactured by electrospinning, a versatile and common technique

used to create fibrous tubular scaffolds (Bhardwaj and Kundu 2010). Biazar et al. reported successful reconstruction of the nerve trunk and myelinated nerve fibers in nanofibrous PHBV electrospun conduits across a 30 mm rat sciatic nerve injury defect (Biazar and Heidari Keshel 2013). Huang et al. fabricated grooved and smooth electrospun cellulose acetate butyrates as electrospun fibrous conduits, which were implanted into a 13 mm rat sciatic nerve injury gap (Huang et al. 2015). Using two different molecular weights, cellulose acetate butyrates resulted in smooth nanofibers and grooved nanofibers, with average fiber size of 500 nm. Overall, grooved nanofibers significantly improved nerve regeneration in a 13 mm rat sciatic nerve injury model compared to smooth fiber conduits (Huang et al. 2015).

5.6 Intraluminal Guidance

The addition of intraluminal guidance scaffolds, such as electrospun fibers, is an increasingly attractive approach for improving nerve guide conduits by introducing topographic cues for regenerating axons (Behbehani et al. 2018). It has been reported that aligned electrospun fibers in the lumen of NGCs act as an additional anchor for the fibrin cable that forms during nerve regeneration inside a tube, and mimics the ECM matrix fibrils. This serves to increase Schwann cell attachment and migration along the fibers and fibrin cable (Daly et al. 2012; Gupta et al. 2009). Two different variables are currently being investigated for the use of electrospun fibers in nerve tissue engineering, and concern fiber alignment and fiber diameter. Chew et al. investigated the effect of Schwann cell phenotype and gene expression on random and aligned 1 μm PCL fibers. Aligned fibers promoted Schwann cell attachment, elongation and a mature phenotype with the upregulation of protein zero (PO) and myelin-associated glycoprotein (MAG, Siglec-4) gene expression (Chew et al. 2008). Kim et al. manufactured random and aligned poly(acrylonitrile-co-methylacrylate) (PAN-MA) fiber films and investigated the in vitro and in vivo effect of fiber orientation on rat pup DRG neurons in a 17 mm rat tibial nerve defect injury model (Kim et al. 2008). Aligned fiber films promoted significantly longer neurite outgrowth and Schwann cell migration from DRG explants, compared to random fiber films, and significantly promoted nerve regeneration in a 17 mm injury gap, compared to random fiber film conduits (Kim et al. 2008). Both these studies highlight the importance of fiber alignment in peripheral nerve repair, promoting a mature Schwann cell phenotype, Schwann cell migration, and neurite outgrowth (Chew et al. 2008; Kim et al. 2008).

The diameter of fibers in a guidance scaffold also plays a significant role in nerve repair. Gnani et al. fabricated 0.3 μm , 0.6 μm , 1 μm , and 1.3 μm gelatin fibers via electrospinning and reported increased neurite outgrowth and primary Schwann cell migration from DRG explants on the larger fibers, 1 and 1.3 μm , compared to the 0.3 and 0.6 μm fibers (Gnani et al. 2015).

Daud et al. fabricated parallel aligned 1, 5, and 8 μm fibers from PCL (80 kDa) by electrospinning and compared the cellular response of NG108-15 neuronal cells, primary Schwann cells, and DRG explants. Overall, 8 μm diameter aligned fibers

supported longer neuronal neurite lengths and Schwann cell attachment. Smaller 1 μm fibers supported Schwann cell attachment, but while effective at supporting initial neuronal cell attachment, were not able to support survival when neuronal cells were cultured alone. In contrast, coculture with primary Schwann cells supported significantly longer neurites and survival of both cell types, supporting a hypothesis of soluble factor local paracrine signaling. When DRG neurite outgrowth length and Schwann cell migration was studied from explants, migration distances of >2.5 mm were observed in vitro for 1, 5, and 8 μm diameter fibers (Daud et al. 2012). Lizarraga-Valderrama et al. manufactured 2.4, 3.7, and 13.5 μm aligned electrospun fibers from a novel PHA blend of 25:75 P(3HO)/P(3HB). The large 13.5 μm diameter fibers supported adhesion of NG108-15 neuronal cells and promoted neuronal cell differentiation (Lizarraga-Valderrama et al. 2019). In summary, the above studies highlight the importance of fiber alignment and the influence of fiber diameter on neuronal and Schwann cell responses for guiding neural regeneration peripheral nerve repair.

5.7 Coatings and Surface Chemical Modification

The surface chemistry of a biomaterial can be employed to influence and improve cellular adhesion, spreading, and migration, as a means to improve overall biocompatibility (Bell and Haycock 2012). This can be adopted in the form of nerve guide “coatings” as a means to improving nerve guide conduits. The use of ECM proteins as coatings, such as collagen and laminin, is a popular approach to improve biocompatibility of bio-inert synthetic polymers. Lee et al. reported that collagen-coated PLGA tubes promoted nerve regeneration more efficiently in a 15 mm rabbit peroneal nerve defect compared with a collagen filled autologous vein graft (Lee et al. 2006). Armstrong et al. investigated the effects of laminin, fibronectin, and collagen on NG108-15 neuronal cell differentiation and Schwann cell proliferation (Armstrong et al. 2007). Overall, neurite outgrowth from NG108-15 neuronal cells was significantly higher when cultured on P(3HB) films coated with laminin and fibronectin, compared to collagen, when cocultured with primary Schwann cells. Laminin-coated P(3HB) films also significantly increased Schwann cell proliferation (Armstrong et al. 2007). Natural coatings improve biocompatibility of nerve guides by mimicking the ECM, providing biochemical ligands for different cell types. However, one must be cautious of potential immune responses and batch to batch variability when considering these approaches (Daly et al. 2012). Therefore, the use of synthetic coatings has a distinct advantage in this regard, by mitigating undesirable immune responses. Synthetic coatings are also cost effective, quality controllable, and can be manufactured in a scalable way (Nectow et al. 2012). Applying synthetic coatings, such as different chemical reactive groups, can be achieved by plasma polymerization, chemical graft modification, and self-assembled monolayer techniques (Thevenot et al. 2008). Plasma polymerization is a reproducible technique to graft chemical reactive groups, such as methyl and amine groups, onto biomaterial surfaces, without changing the bulk properties of

the biomaterial (Chua et al. 2002). Different chemical groups have been investigated for their effect on neuronal cells using plasma polymerization. Buttiglione et al. grafted amine groups on carboxylic acid groups onto polyethylene terephthalate (PET) surfaces by plasma polymerization, using allylamine and acrylic acid respectively (Buttiglione et al. 2007). The addition of amine groups increased SH-SY5Y neuroblastoma cell proliferation and adhesion onto PET surfaces, whereas carboxyl groups increased SH-SY5Y neurofilament-200 expression, and neuronal cell differentiation (Buttiglione et al. 2007).

5.8 Incorporation of Growth Factors

After nerve injury, the secretion of growth factors from Bünger Schwann cells is vital for nerve regeneration, stimulating nerve growth cones from the proximal stump (Deumens et al. 2010). Growth factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and glial-derived neurotrophic factor (GDNF), are important for controlling cell proliferation, differentiation, morphogenesis, and apoptosis (Li et al. 2020). Incorporating growth factors into nerve guide conduits and/or into intraluminal guidance scaffolds is another approach for improving nerve regeneration (Pfister et al. 2007). Liu et al. developed core-shelled conduits, fabricated by coaxial electrospinning, in which poly(lactic acid-caprolactone) (P(LLA-CL)) was used to make the protective outer shell, and solutions of NGF-bovine serum albumin (BSA) in PBS spun into the core of the conduit (Liu et al. 2011). In a 10 mm rat sciatic nerve injury defect, similar nerve regeneration was observed in both P(LLA-CL)/NGF conduits and the autograft control, with regard to regenerated nerve fibers, myelinated fibers, and overall nerve function (Kalbermatten et al. 2008). Nerve regeneration was significantly greater in both P(LLA-CL)/NGF conduits and the autograft control compared to empty P(LLA-CL) conduits and NGF injected P(LLA-CL) conduits (Liu et al. 2011). Terris et al. manufactured cross-linked collagen tubules, and implanted collagen gel filled tubules, BDNF enriched gel filled tubules, and reversed nerve grafts into a 15 mm rabbit facial defect model (Terris et al. 2001). Functional nerve recovery was similar for all three samples, and although mean axonal diameter and axon count were highest in the BDNF-enriched gel-filled collagen tubes, statistical differences were not detected (Terris et al. 2001). GDNF has also been incorporated into porous PCL conduits and implanted into a 5 cm median nerve defect in a nonhuman primate model in the study reported by Fadia et al. (Fadia et al. 2020). Similar functional recovery was observed between PCL/GDNF conduits and the autograft control, and PCL/GDNF conduits promoted increased Schwann cell migration and axonal regeneration, occupying a significantly larger area of Schwann cells at the distal stump, compared to the other groups (Fadia et al. 2020). These studies highlight the increase in nerve regeneration by incorporating growth factors into nerve guide conduits compared to synthetic conduits alone, and the release of growth factors locally can be controlled over time to prolong their delivery and stimulate nerve regeneration more effectively during wound healing and repair (Carvalho et al. 2019).

5.9 Use of Supporting Cells

The addition of supporting cells to nerve guide conduits is an advantageous method to improving nerve guide conduits. Schwann cells are the most common cell type to use in regard to nerve regeneration, as they form the Bands of Bünger, secrete neurotrophic factors to promote nerve regeneration, and myelinate the regenerating axons (Deumens et al. 2010). Schwann cells can be introduced to nerve guide conduits by injection, seeded onto intraluminal guidance scaffolds, and released from the tubular construct. Several sources of Schwann cells have been investigated (Daly et al. 2012). Hadlock et al. fabricated porous PLGA conduits, using a novel foam processing technique, and primary Schwann cells were seeded onto conduits (Hadlock et al. 2000). Using a 7 mm rat sciatic nerve injury model, axonal regeneration was observed in the middle of Schwann cell seeded PLGA conduits. After 6 weeks' implantation, the percentage of neuronal tissue was similar to the autograft control, although mean axon diameter was significantly lower compared to the autograft control (Hadlock et al. 2000). Georgiou et al. fabricated engineered neural tissue (EngNT) constructs, seeding SCL 4.1/F7 Schwann cells into collagen, and fabricated tethered aligned collagen gel sheets (Georgiou et al. 2013). Conduits were implanted into a 15 mm rat sciatic nerve defect for 8 weeks as well as an autograft control and Neurawrap™ tubes, with FDA approved collagen NGC for comparison. However, the autograft control was superior compared to Neurawrap™ tube in regard to myelin diameter and axon diameter, and superior to both the EngNT tubes and Neurawrap™ tube with regard to myelin thickness (Georgiou et al. 2013). In contrast, axonal regeneration in the distal part of the conduit and stump was significantly higher in the EngNT tubes compared to the Neurawrap™ tube (Georgiou et al. 2013).

The source of Schwann cells for such therapies remains a challenge. The painful isolation procedures with concomitant damage for obtaining autologous Schwann cells, and the use of immunosuppressive drugs when considering allogenic Schwann cell use, plus costs associated with cell expansion, introduce complexities that must be carefully balanced with clinical need and effectiveness beyond cell-free NGC approaches (Daly et al. 2012; Mosahebi et al. 2002). Therefore, alternative sources of Schwann cells, such as those derived from stem cells, offer an alternative approach. Schwann cells can be derived from neural, embryonic, bone mesenchymal, and adipose derived stem cells (Bell and Haycock 2012). Kalbermatten et al. fabricated conduits by rolling P(3HB) sheets and incorporated primary Schwann cells, differentiated mesenchymal stem cells (dMSCs), and undifferentiated mesenchymal stem cells (uMSCs) into conduits by suspending cells in an internal fibrin matrix (Kalbermatten et al. 2008). In vitro analysis confirmed significantly lower cell numbers and cell distribution in the fibrin matrix using uMSCs compared to dMSCs and Schwann cells, and so uMSCs were not analyzed in vivo. In vivo analysis, in a 10 mm rat sciatic nerve injury model, showed after 2 weeks' implantation that Schwann cell and axon regeneration distances were superior using fibrin matrix incorporated Schwann cells and differentiated mesenchymal stem cells (dMSCs), compared to empty conduits and fibrin matrix- (without cell) filled conduits

(Kalbermatten et al. 2008). Overall, conduits filled with the Schwann cell incorporated fibrin matrix promoted enhanced nerve regeneration (Kalbermatten et al. 2008). However, the use of mesenchymal stem cells (MSCs) clinically is not a popular method for peripheral nerve repair, as isolation techniques are painful for the patient (Bell and Haycock 2012). In contrast, the use of adipose derived stem cells (ADSCs) is more amenable as ADSCs can be isolated and harvested from patient lipoaspirate and can produce a high stem yield of 2% from the total cell adipose yield (Kingham et al. 2007). ADSCs have been shown to differentiate into Schwann-like cells and have been incorporated into nerve guides for peripheral nerve repair (Kaewkhaw et al. 2011). Di Summa et al. fabricated nerve conduits from fibrin and before *in vivo* implantation were injected with primary Schwann cells or Schwann like cells differentiated from ADSCs and MSCs (Summa et al. 2010). Cellular conduits were implanted into a 10 mm rat sciatic nerve defect for 16 weeks. Overall, axonal regeneration and Schwann cell migration to the distal stump were significantly greater in cellular conduits compared to the empty conduit, in which SC seeded conduits were the most efficient out of the cellular conduits, though not significantly more efficient (Summa et al. 2010). Similar results were observed between the ADSCs and MSCs seeded conduits, and overall, due to ease of isolation, the use of ADSCs would be preferable (Bell and Haycock 2012).

6 Conclusions

This chapter highlights the amount of research being performed in the area of biomaterials for peripheral nerve repair and nerve regeneration. We critique the importance of biomaterial choice for fabricating nerve guide conduits. It summarizes major recent studies with regard to specific criteria that must be met to provide optimal nerve regeneration and repair, including bulk chemistry, surface chemistry, degradation rates, biocompatibility, porosity, and mechanical properties (Kehoe et al. 2012). Natural and synthetic materials are compared, with respective advantages and disadvantages. The blending of natural and synthetic materials together to form composite conduits is seen as an emerging route for overcoming disadvantages associated with both biomaterial types. An emerging number of different manufacturing methods are being reported that can process natural and synthetic materials into NGCs, incorporating new designs and with intricate detail. Furthermore, there are many additional approaches to improving hollow NGCs, such as the use of intraluminal guidance scaffolds, modifying the surfaces of biomaterials, and the addition of growth factors and support cells to increase Schwann cell migration and axonal regeneration from the proximal to the distal nerve stump. Overall, a combinatorial approach, using a guidance scaffold plus a growth factor, for example, is proving to be an effective approach for improving current hollow NGCs. Although further investigation is still required, researchers are getting closer to fabricating nerve guide conduits that are comparable to the gold standard of autograft for repair of short to medium traumatic injury gaps.

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References

- Armstrong SJ, Wiberg M, Terenghi G, Kingham PJ (2007) ECM molecules mediate both Schwann cell proliferation and activation to enhance neurite outgrowth. *Tissue Eng* 13(12):2863–2870
- Arslantunali D, Dursun T, Yucel D, Hasirci N, Hasirci V (2014) Peripheral nerve conduits: technology update. *Med Devices (Auckl)* 7:405–424
- Assaf K, Leal CV, Derami MS, de Rezende Duek EA, Ceragioli HJ, de Oliveira ALR (2017) Sciatic nerve repair using poly(ϵ -caprolactone) tubular prosthesis associated with nanoparticles of carbon and graphene. *Brain Behav* 7(8):e00755
- Bak M, Gutkowska ON, Wagner E, Gosk J (2017) The role of chitin and chitosan in peripheral nerve reconstruction. *Polim Med* 47(1):43–47
- Basnett P, Ching KY, Stolz M, Knowles JC, Boccaccini AR, Smith C, Locke IC, Keshavarz T, Roy I (2013) Novel Poly(3-hydroxyoctanoate)/Poly(3-hydroxybutyrate) blends for medical applications. *React Funct Polym* 73(10):1340–1348
- Baumann N, Pham-Dinh D (2001) Biology of oligodendrocyte and myelin in the mammalian central nervous system. *Physiol Rev* 81(2):871–927
- Behbehani M, Glen A, Taylor CS, Schuhmacher A, Claeysens F, Haycock JW (2018) Pre-clinical evaluation of advanced nerve guide conduits using a novel 3D in vitro testing model. *Int J Bioprint* 4(1):1–13
- Belkas JS, Shoichet MS, Midha R (2004) Axonal guidance channels in peripheral nerve regeneration. *Oper Tech Orthop* 14(3):190–198
- Bell JH, Haycock JW (2012) Next generation nerve guides: materials, fabrication, growth factors, and cell delivery. *Tissue Eng Part B Rev* 18(2):116–128
- Bhardwaj N, Kundu SC (2010) Electrospinning: a fascinating fiber fabrication technique. *Biotechnol Adv* 28(3):325–347
- Biazar E, Heidari Keshel S (2013) A nanofibrous PHBV tube with Schwann cell as artificial nerve graft contributing to rat sciatic nerve regeneration across a 30-mm defect bridge. *Cell Commun Adhes* 20(1–2):41–49
- Biazar E, Keshel SH (2013) Chitosan–cross-linked nanofibrous PHBV nerve guide for rat sciatic nerve regeneration across a defect bridge. *ASAIO J* 59(6):651–659
- Boecker A, Daeschler SC, Kneser U, Harhaus L (2019) Relevance and recent developments of chitosan in peripheral nerve surgery. *Front Cell Neurosci* 13:104
- Boni R, Ali A, Shavandi A, Clarkson AN (2018) Current and novel polymeric biomaterials for neural tissue engineering. *J Biomed Sci* 25(1):90
- Bozkurt A, van Neerven SGA, Claeys KG, O'Dey DM, Sudhoff A, Brook GA, Sellhaus B, Schulz JB, Weis J, Pallua N (2014) The proximal medial sural nerve biopsy model: a standardised and reproducible baseline clinical model for the translational evaluation of bioengineered nerve guides. *BioMed Res Int* 2014:121452–121463
- Bozkurt A, Claeys KG, Schrading S, Rödler JV, Altinova H, Schulz JB, Weis J, Pallua N, van Neerven SGA (2017) Clinical and biometrical 12-month follow-up in patients after reconstruction of the sural nerve biopsy defect by the collagen-based nerve guide Neuromaix. *Eur J Med Res* 22(1):22–34
- Buttiglione M, Vitiello F, Sardella E, Petrone L, Nardulli M, Favia P, d'Agostino R, Gristina R (2007) Behaviour of SH-SY5Y neuroblastoma cell line grown in different media and on different chemically modified substrates. *Biomaterials* 28(19):2932–2945

- Carvalho CR, Oliveira JM, Reis RL (2019) Modern trends for peripheral nerve repair and regeneration: beyond the hollow nerve guidance conduit. *Front Bioeng Biotechnol* 7(337):1–30
- Casalini T, Rossi F, Castrovinci A, Perale G (2019) A perspective on polylactic acid-based polymers use for nanoparticles synthesis and applications. *Front Bioeng Biotechnol* 7:259
- Chen R, Hunt JA, Fawcett S, D'sa R, Akhtar R, Curran JM (2018) The optimization and production of stable homogeneous amine enriched surfaces with characterized nanotopographical properties for enhanced osteoinduction of mesenchymal stem cells. *J Biomed Mater Res A* 106(7):1862–1877
- Chew SY, Mi R, Hoke A, Leong KW (2008) The effect of the alignment of electrospun fibrous scaffolds on Schwann cell maturation. *Biomaterials* 29(6):653–661
- Chu PK, Chena JY, Wanga LP, Huang N (2002) Plasma-surface modification of biomaterials. *Mater Sci Eng* 36:143–206
- Daly W, Yao L, Zeugolis D, Windebank A, Pandit A (2012) A biomaterials approach to peripheral nerve regeneration: bridging the peripheral nerve gap and enhancing functional recovery. *J R Soc Interface* 9(67):202–221
- Daly WT, Knight AM, Wang H, de Boer R, Giusti G, Dadsetan M, Spinner RJ, Yaszemski MJ, Windebank AJ (2013) Comparison and characterization of multiple biomaterial conduits for peripheral nerve repair. *Biomaterials* 34(34):8630–8639
- Daud MF, Pawar KC, Claeysens F, Ryan AJ, Haycock JW (2012) An aligned 3D neuronal-glial coculture model for peripheral nerve studies. *Biomaterials* 33(25):5901–5913
- de Luca AC, Lacour SP, Raffoul W, di Summa PG (2014) Extracellular matrix components in peripheral nerve repair: how to affect neural cellular response and nerve regeneration? *Neural Regen Res* 9(22):1943–1948
- de Ruiter GCW, Malessy MJA, Yaszemski MJ, Windebank AJ, Spinner RJ (2009) Designing ideal conduits for peripheral nerve repair. *Neurosurg Focus* 26(2):E5
- Dellon AL, Mackinnon SE (1988) An alternative to the classical nerve graft for the management of the short nerve gap. *Plast Reconstr Surg* 82(5):849–856
- Denis P, Wrzecionek M, Gadomska-Gajadur A, Sajkiewicz P (2019) Poly(Glycerol Sebacate)–Poly(L-Lactide) nonwovens. Towards attractive electrospun material for tissue engineering. *Polymers* 11(12):1–26
- Deumens R, Bozkurt A, Meek MF, Marcus MA, Joosten EA, Weis J, Brook GA (2010) Repairing injured peripheral nerves: bridging the gap. *Prog Neurobiol* 92(3):245–276
- di Summa PG, Kingham PJ, Raffoul W, Wiberg M, Terenghi G, Kalbermatten DF (2010) Adipose-derived stem cells enhance peripheral nerve regeneration. *J Plast Reconstr Aesthet Surg* 63(9):1544–1552
- Diez-Ahedo R, Mendibil X, Márquez-Posadas CM, Quintana I, González F, Rodríguez JF, Zilic L, Sherborne C, Glen A, Taylor SC, Claeysens F, Haycock WJ, Schaafsma W, González E, Castro B, Merino S (2020) UV-casting on methacrylated PCL for the production of a peripheral nerve implant containing an array of porous aligned microchannels. *Polymers* 12(4):1–19
- Duffy P, McMahon S, Wang X, Keaveney S, O'Cearbhaill ED, Quintana I, Rodríguez FJ, Wang W (2019) Synthetic bioresorbable poly- α -hydroxyesters as peripheral nerve guidance conduits; a review of material properties, design strategies and their efficacy to date. *Biomater Sci* 7(12):4912–4943
- Ebrahimi M, Ai J, Biazar E, Ebrahimi-Barough S, Khojasteh A, Yazdankhah M, Sharifi S, Ai A, Heidari-Keshel S (2018) In vivo assessment of a nanofibrous silk tube as nerve guide for sciatic nerve regeneration. *Artif Cells Nanomed Biotechnol* 46(sup1):394–401
- Elsawy MA, Kim K-H, Park J-W, Deep A (2017) Hydrolytic degradation of polylactic acid (PLA) and its composites. *Renew Sust Energ Rev* 79:1346–1352
- Evans GRD, Brandt K, Widmer MS, Lu L, Meszlenyi RK, Gupta PK, Mikos AG, Hodges J, Williams J, Gürlek A, Nabawi A, Lohman R, Patrick CW (1999) In vivo evaluation of poly(l-lactic acid) porous conduits for peripheral nerve regeneration. *Biomaterials* 20(12):1109–1115
- Fadia NB, Bliley JM, DiBernardo GA, Crammond DJ, Schilling BK, Sivak WN, Spiess AM, Washington KM, Waldner M, Liao H-T, James IB, Minter DM, Tompkins-Rhoades C,

- Cottrill AR, Kim D-Y, Schweizer R, Bourne DA, Panagis GE, Asher Schusterman M, Egro FM, Campwala IK, Simpson T, Weber DJ, Gause T, Brooker JE, Josyula T, Guevara AA, Repko AJ, Mahoney CM, Marra KG (2020) Long-gap peripheral nerve repair through sustained release of a neurotrophic factor in nonhuman primates. *Sci Transl Med* 12(527):eaav7753
- Field J (2019) Manufacture, characterisation and in vivo assessment of nerve guidance conduits containing physical guidance cues. PhD thesis, White Rose Etheses Online, University of Sheffield
- Freier T, Montenegro R, Shan Koh H, Shoichet MS (2005) Chitin-based tubes for tissue engineering in the nervous system. *Biomaterials* 26(22):4624–4632
- Frost HK, Andersson T, Johansson S, Englund-Johansson U, Ekström P, Dahlin LB, Johansson F (2018) Electrospun nerve guide conduits have the potential to bridge peripheral nerve injuries in vivo. *Nat Sci Rep* 8(1):16716
- Gentile P, Chiono V, Carmagnola I, Hatton PV (2014) An overview of poly(lactic-co-glycolic) acid (PLGA)-based biomaterials for bone tissue engineering. *Int J Mol Sci* 15(3):3640–3659
- Georgiou M, Bunting SCJ, Davies HA, Loughlin AJ, Golding JP, Phillips JB (2013) Engineered neural tissue for peripheral nerve repair. *Biomaterials* 34(30):7335–7343
- Ghafaralahi S, Ebrahimian-Hosseiniabadi M, Zargar Kharazi A (2018) Poly(glycerol-sebacate)/poly(caprolactone)/graphene nanocomposites for nerve tissue engineering. *J Bioact Compat Polym* 33(5):529–542
- Gnavi S, Fornasari BE, Tonda-Turo C, Ciardelli G, Zanetti M, Geuna S, Perroteau I (2015) The influence of electrospun fibre size on Schwann cell behaviour and axonal outgrowth. *Mater Sci Eng Part C* 48:620–631
- Gupta D, Venugopal J, Prabhakaran MP, Dev VR, Low S, Choon AT, Ramakrishna S (2009) Aligned and random nanofibrous substrate for the in vitro culture of Schwann cells for neural tissue engineering. *Acta Biomater* 5(7):2560–2569
- Hadlock T, Sundback C, Hunter D, Cheney M, Vacanti JP (2000) A polymer foam conduit seeded with Schwann cells promotes guided peripheral nerve regeneration. *Tissue Eng* 6(2):119–127
- Hashimoto T, Suzuki Y, Kitada M, Kataoka K, Wu S, Suzuki K, Endo K, Nishimura Y, Ide C (2002) Peripheral nerve regeneration through alginate gel: analysis of early outgrowth and late increase in diameter of regenerating axons. *Exp Brain Res* 146(3):356–368
- Hazari A, Wiberg M, Johansson-Rudén G, Green C, Terenghi G (1999) A resorbable nerve conduit as an alternative to nerve autograft in nerve gap repair. *Br J Plast Surg* 52(8):653–657
- Hazer DB, Bal E, Nurlu G, Benli K, Balci S, Öztürk F, Hazer B (2013) In vivo application of poly-3-hydroxyoctanoate as peripheral nerve graft. *J Zhejiang Univ Sci B* 14(11):993–1003
- Holland C, Numata K, Rnjak-Kovacina J, Seib FP (2019) The biomedical use of silk: past, present, future. *Adv Healthc Mater* 8(1):1800465
- Huang W, Begum R, Barber T, Ibba V, Tee NCH, Hussain M, Arastoo M, Yang Q, Robson LG, Lesage S, Gheysens T, Skaer NJV, Knight DP, Priestley JV (2012) Regenerative potential of silk conduits in repair of peripheral nerve injury in adult rats. *Biomaterials* 33(1):59–71
- Huang C, Ouyang Y, Niu H, He N, Ke Q, Jin X, Li D, Fang J, Liu W, Fan C, Lin T (2015) Nerve guidance conduits from aligned nanofibers: improvement of nerve regeneration through longitudinal nanogrooves on a fiber surface. *ACS Appl Mater Interfaces* 7(13):7189–7196
- Huxley AF, Stämpeli R (1949) Evidence for saltatory conduction in peripheral myelinated nerve fibres. *J Physiol* 108(3):315–339
- Jansen K, van der Werff JFA, van Wachem PB, Nicolai JPA, de Leij LFMH, van Luyn MJA (2004) A hyaluronan-based nerve guide: in vitro cytotoxicity, subcutaneous tissue reactions, and degradation in the rat. *Biomaterials* 25(3):483–489
- Jiang X, Lim SH, Mao H-Q, Chew SY (2010) Current applications and future perspectives of artificial nerve conduits. *Exp Neurol* 223(1):86–101
- Johnson DW, Sherborne C, Didsbury MP, Pateman C, Cameron NR, Claeysens F (2013) Macrostructuring of emulsion-templated porous polymers by 3D laser patterning. *Adv Mater* 25(23):3178–3181

- Kaewkhaw R, Scutt AM, Haycock JW (2011) Anatomical site influences the differentiation of adipose-derived stem cells for Schwann-cell phenotype and function. *Glia* 59(5):734–749
- Kalbermatten DF, Kingham PJ, Mahay D, Mantovani C, Pettersson J, Raffoul W, Balcin H, Pierer G, Terenghi G (2008) Fibrin matrix for suspension of regenerative cells in an artificial nerve conduit. *J Plast Reconstr Aesthet Surg* 61(6):669–675
- Karimi M, Biazar E, Keshel SH, Ronaghi A, Doostmohamadpour J, Janfada A, Montazeri A (2014) Rat Sciatic Nerve Reconstruction Across a 30 mm Defect Bridged by an Oriented Porous PHBV Tube With Schwann Cell as Artificial Nerve Graft. *ASAIO* 60(2):224–233
- Kehoe S, Zhang XF, Boyd D (2012) FDA approved guidance conduits and wraps for peripheral nerve injury: a review of materials and efficacy. *Injury* 43(5):553–572
- Kim YT, Haftel VK, Kumar S, Bellamkonda RV (2008) The role of aligned polymer fiber-based constructs in the bridging of long peripheral nerve gaps. *Biomaterials* 29(21):3117–3127
- Kingham PJ, Kalbermatten DF, Mahay D, Armstrong SJ, Wiberg M, Terenghi G (2007) Adipose-derived stem cells differentiate into a Schwann cell phenotype and promote neurite outgrowth in vitro. *Exp Neurol* 207(2):267–274
- Koh L-D, Cheng Y, Teng C-P, Khin Y-W, Loh X-J, Tee S-Y, Low M, Ye E, Yu H-D, Zhang Y-W, Han M-Y (2015) Structures, mechanical properties and applications of silk fibroin materials. *Prog Polym Sci* 46:86–110
- Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. *Prog Polym Sci* 37(1):106–126
- Lee D-Y, Choi B-H, Park J-H, Zhu S-J, Kim B-Y, Huh J-Y, Lee S-H, Jung J-H, Kim S-H (2006) Nerve regeneration with the use of a poly(l-lactide-co-glycolic acid)-coated collagen tube filled with collagen gel. *J Cranio-Maxillofac Surg* 34(1):50–56
- Li S, Wu H, Hu X-D, Tu C-Q, Pei F-X, Wang G-L, Lin W, Fan H-S (2012) Preparation of electrospun PLGA-silk fibroin nanofibers-based nerve conduits and evaluation in vivo. *Artif Cells Blood Substit Biotechnol* 40(1–2):171–178
- Li R, Liu H, Huang H, Bi W, Yan R, Tan X, Wen W, Wang C, Song W, Zhang Y, Zhang F, Hu M (2018) Chitosan conduit combined with hyaluronic acid prevent sciatic nerve scar in a rat model of peripheral nerve crush injury. *Mol Med Rep* 17(3):4360–4368
- Li R, Li D-h, Zhang H-y, Wang J, Li X-k, Xiao J (2020) Growth factors-based therapeutic strategies and their underlying signaling mechanisms for peripheral nerve regeneration. *Acta Pharmacologica Sinica* 0:1–12
- Liou H-M, Rau L-R, Huang C-C, Lu M-R, Hsu F-Y (2013) Electrospun hyaluronan-gelatin nanofibrous matrix for nerve tissue engineering. *J Nanomater* 2013:613638
- Liu J-J, Wang C-Y, Wang J-G, Ruan H-J, Fan C-Y (2011) Peripheral nerve regeneration using composite poly(lactic acid-caprolactone)/nerve growth factor conduits prepared by coaxial electrospinning. *J Biomed Mater Res A* 96A(1):13–20
- Liu T, Houle JD, Xu J, Chan BP, Chew SY (2012) Nanofibrous collagen nerve conduits for spinal cord repair. *Tissue Eng A* 18(9–10):1057–1066
- Lizarraga-Valderrama LR, Nigmatullin R, Taylor C, Haycock JW, Claeysens F, Knowles JC, Roy I (2015) Nerve tissue engineering using blends of poly(3-hydroxyalkanoates) for peripheral nerve regeneration. *Eng Life Sci* 15(6):612–621
- Lizarraga-Valderrama LR, Panchal B, Thomas C, Boccaccini AR, Roy I (2016) Biomedical applications of polyhydroxyalkanoates, biomaterials from nature for advanced devices and therapies, pp 337–383
- Lizarraga-Valderrama LR, Taylor CS, Claeysens F, Haycock JW, Knowles JC, Roy I (2019) Unidirectional neuronal cell growth and differentiation on aligned polyhydroxyalkanoate blend microfibres with varying diameters. *J Tissue Eng Regen Med* 13(9):1581–1594
- Loh XJ, Abdul Karim A, Ow C (2015) Poly(glycerol sebacate) biomaterial: synthesis and biomedical applications. *J Mater Chem B* 3(39):7641–7652
- Lowe JS, Anderson PG (2015) Chapter 6: Nervous tissue. In: Stevens & Lowe's human histology, 4th edn. Published by Mosby Ltd, pp 84–104

- Magaz A, Faroni A, Gough JE, Reid AJ, Li X, Blaker JJ (2018) Bioactive silk-based nerve guidance conduits for augmenting peripheral nerve repair. *Adv Healthc Mater* 7(23):1800308
- Marani E, Lakke EAJF, Paxinos G (2012) Chapter 4: Peripheral nervous system topics A2. In: Mai JK (ed) *The human nervous system*, 3rd edn, Published by Academic Press, Elsevier, pp 82–140
- Meyer C, Stenberg L, Gonzalez-Perez F, Wrobel S, Ronchi G, Udina E, Suganuma S, Geuna S, Navarro X, Dahlin LB, Grothe C, Haastert-Talini K (2016) Chitosan-film enhanced chitosan nerve guides for long-distance regeneration of peripheral nerves. *Biomaterials* 76:33–51
- Mobasserri A, Faroni A, Minogue BM, Downes S, Terenghi G, Reid AJ (2015) Polymer scaffolds with preferential parallel grooves enhance nerve regeneration. *Tissue Eng A* 21 (5–6):1152–1162
- Mosahebi A, Fuller P, Wiberg M, Terenghi G (2002) Effect of allogeneic Schwann cell transplantation on peripheral nerve regeneration. *Exp Neurol* 173(2):213–223
- Mosahebi A, Wiberg M, Terenghi G (2003) Addition of fibronectin to alginate matrix improves peripheral nerve regeneration in tissue-engineered conduits. *Tissue Eng* 9(2):209–218
- Nectow AR, Marra KG, Kaplan DL (2012) Biomaterials for the development of peripheral nerve guidance conduits. *Tissue Eng Part B Rev* 18(1):40–50
- Neubrech F, Heider S, Harhaus L, Bickert B, Kneser U, Kremer T (2016) Chitosan nerve tube for primary repair of traumatic sensory nerve lesions of the hand without a gap: study protocol for a randomized controlled trial. *Trials* 17:48–48
- Oh SH, Kim JH, Song KS, Jeon BH, Yoon JH, Seo TB, Namgung U, Lee IW, Lee JH (2008) Peripheral nerve regeneration within an asymmetrically porous PLGA/Pluronic F127 nerve guide conduit. *Biomaterials* 29(11):1601–1609
- Oh SH, Kim JR, Kwon GB, Namgung U, Song KS, Lee JH (2013) Effect of surface pore structure of nerve guide conduit on peripheral nerve regeneration. *Tissue Eng Part C Methods* 19(3):233–243
- Okamoto H, Hata K-I, Kagami H, Okada K, Ito Y, Narita Y, Hirata H, Sekiya I, Otsuka T, Ueda M (2010) Recovery process of sciatic nerve defect with novel bioabsorbable collagen tubes packed with collagen filaments in dogs. *J Biomed Mater Res A* 92A(3):859–868
- Owen R, Sherborne C, Paterson T, Green NH, Reilly GC, Claeysens F (2016) Emulsion templated scaffolds with tunable mechanical properties for bone tissue engineering. *J Mech Behav Biomed Mater* 54:159–172
- Pateman CJ, Harding AJ, Glen A, Taylor CS, Christmas CR, Robinson PP, Rimmer S, Boissonade FM, Claeysens F, Haycock JW (2015) Nerve guides manufactured from photocurable polymers to aid peripheral nerve repair. *Biomaterials* 49:77–89
- Paxinos G, Xu-Feng H, Sengul G, Watson C (2012) Chapter 8: Organization of Brainstem nuclei. In: *The human nervous system*, 3rd edn. Published by Academic press, Elsevier, pp 260–327
- Perry VH, Brown MC, Gordon S (1987) The macrophage response to central and peripheral nerve injury. A possible role for macrophages in regeneration. *J Exp Med* 165(4):1218–1223
- Pfister LA, Papaloizos M, Merkle HP, Gander B (2007) Nerve conduits and growth factor delivery in peripheral nerve repair. *J Peripher Nerv Syst* 12(2):65–82
- Pfister BJ, Gordon T, Loverde JR, Kochar AS, Mackinnon SE, Cullen DK (2011) Biomedical engineering strategies for peripheral nerve repair: surgical applications, state of the art, and future challenges. *Crit Rev Biomed Eng* 39(2):81–124
- Philip S, Keshavarz T, Roy I (2007) Polyhydroxyalkanoates: biodegradable polymers with a range of applications. *J Chem Technol Biotechnol* 82(3):233–247
- Phillips JB, Bunting SCJ, Hall SM, Brown RA (2005) Neural tissue engineering: a self-organizing collagen guidance conduit. *Tissue Eng* 11(9–10):1611–1617
- Rai R, Tallawi M, Grigore A, Boccaccini AR (2012) Synthesis, properties and biomedical applications of poly(glycerol sebacate) (PGS): a review. *Prog Polym Sci* 37(8):1051–1078
- Reid AJ, de Luca AC, Faroni A, Downes S, Sun M, Terenghi G, Kingham PJ (2013) Long term peripheral nerve regeneration using a novel PCL nerve conduit. *Neurosci Lett* 544:125–130

- Rodríguez-Vázquez M, Vega-Ruiz B, Ramos-Zúñiga R, Saldaña-Koppel DA, Quiñones-Olvera LF (2015) Chitosan and its potential use as a scaffold for tissue engineering in regenerative medicine. *Biomed Res Int* 2015:821279–821279
- Rosson GD, Williams EH, Dellon AL (2009) Motor nerve regeneration across a conduit. *Microsurgery* 29(2):107–114
- Sakai Y, Matsuyama Y, Takahashi K, Sato T, Hattori T, Nakashima S, Ishiguro N (2007) New artificial nerve conduits made with photocrosslinked hyaluronic acid for peripheral nerve regeneration. *Biomed Mater Eng* 17:191–197
- Shen H, Shen ZL, Zhang PH, Chen NL, Wang YC, Zhang ZF, Jin YQ (2010) Ciliary neurotrophic factor-coated polylactic-polyglycolic acid chitosan nerve conduit promotes peripheral nerve regeneration in canine tibial nerve defect repair. *J Biomed Mater Res B Appl Biomater* 95B(1):161–170
- Shen G, Hu X, Guan G, Wang L (2015) Surface modification and characterisation of silk fibroin fabric produced by the layer-by-layer self-assembly of multilayer alginate/regenerated silk fibroin. *PLoS One* 10(4):0124811–0124811
- Singh D, Harding AJ, Albadawi E, Boissonade FM, Haycock JW, Claeysens F (2018) Additive manufactured biodegradable poly(glycerol sebacate methacrylate) nerve guidance conduits. *Acta Biomater* 78:48–63
- Sun J, Tan H (2013) Alginate-based biomaterials for regenerative medicine applications. *Materials* 6(4):1285–1309
- Sun M, Kingham PJ, Reid AJ, Armstrong SJ, Terenghi G, Downes S (2009) In vitro and in vivo testing of novel ultrathin PCL and PCL/PLA blend films as peripheral nerve conduit. *J Biomed Mater Res A* 93A(4):1470–1481
- Sun M, McGowan M, Kingham PJ, Terenghi G, Downes S (2010) Novel thin-walled nerve conduit with microgrooved surface patterns for enhanced peripheral nerve repair. *J Mater Sci Mater Med* 21(10):2765–2774
- Sundback CA, Shyu JY, Wang Y, Faquin WC, Langer RS, Vacanti JP, Hadlock TA (2005) Biocompatibility analysis of poly(glycerol sebacate) as a nerve guide material. *Biomaterials* 26(27):5454–5464
- Suzuki Y, Tanihara M, Ohnishi K, Suzuki K, Endo K, Nishimura Y (1999) Cat peripheral nerve regeneration across 50 mm gap repaired with a novel nerve guide composed of freeze-dried alginate gel. *Neurosci Lett* 259(2):75–78
- Szarek D, Marycz K, Bednarz P, Tabakow P, Jarmundowicz W, Laska J (2013) Influence of calcium alginate on peripheral nerve regeneration: in vivo study. *Biotechnol Appl Biochem* 60(6):547–556
- Terris DJ, Toft KM, Moir M, Lum J, Wang M (2001) Brain-derived neurotrophic factor-enriched collagen tubule as a substitute for autologous nerve grafts. *Arch Otolaryngol Head Neck Surg* 127(3):294–298
- Thevenot P, Hu W, Tang L (2008) Surface chemistry influences implant biocompatibility. *Curr Top Med Chem* 8(4):270–280
- Tokiwa Y, Calabria BP (2007) Biodegradability and biodegradation of polyesters. *J Polym Environ* 15(4):259–267
- Ulery BD, Nair LS, Laurencin CT (2011) Biomedical applications of biodegradable polymers. *J Polym Sci B Polym Phys* 49(12):832–864
- Valappil SP, Misra SK, Boccaccini AR, Roy I (2006) Biomedical applications of polyhydroxyalkanoates: an overview of animal testing and in vivo responses. *Expert Rev Med Devices* 3(6):853–868
- Vasvani S, Kulkarni P, Rawtani D (2019) Hyaluronic acid: a review on its biology, aspects of drug delivery, route of administrations and a special emphasis on its approved marketed products and recent clinical studies. *Int J Biol Macromol* 151:1012–1029
- Verdú E, Labrador RO, Rodríguez FJ, Ceballos D, Forés J, Navarro X (2002) Alignment of collagen and laminin-containing gels improve nerve regeneration within silicone tubes. *Restor Neurol Neurosci* 20(5):169–179

- Waitayawinyu T, Parisi DM, Miller B, Luria S, Morton HJ, Chin SH, Trumble TE (2007) A comparison of polyglycolic acid versus type I collagen bioabsorbable nerve conduits in a rat model: an alternative to autografting. *J Hand Surg Am* 32(10):1521–1529
- Wang S, Cai L (2010) Polymers for fabricating nerve conduits. *Int J Poly Sci* 2010:138686
- Wang W, Itoh S, Konno K, Kikkawa T, Ichinose S, Sakai K, Ohkuma T, Watabe K (2009) Effects of Schwann cell alignment along the oriented electrospun chitosan nanofibers on nerve regeneration. *J Biomed Mater Res A* 91A(4):994–1005
- Wang X, He J, Wang Y, Cui F-Z (2012) Hyaluronic acid-based scaffold for central neural tissue engineering. *Interface focus* 2(3):278–291
- Weber RA, Breidenbach WC, Brown RE, Jabaley ME, Mass DP (2000) A randomized prospective study of polyglycolic acid conduits for digital nerve reconstruction in humans. *Plast Reconstr Surg* 106(5):1036–1045
- Wrobel S, Serra SC, Ribeiro-Samy S, Sousa N, Heimann C, Barwig C, Grothe C, Salgado AJ, Haastert-Talini K (2014) In vitro evaluation of cell-seeded chitosan films for peripheral nerve tissue engineering. *Tissue Eng A* 20(17–18):2339–2349
- Xu X-Y, Li X-T, Peng S-W, Xiao J-F, Liu C, Fang G, Chen KC, Chen G-Q (2010) The behaviour of neural stem cells on polyhydroxyalkanoate nanofiber scaffolds. *Biomaterials* 31(14):3967–3975
- Yang Y, De Laporte L, Rives CB, Jang J-H, Lin W-C, Shull KR, Shea LD (2005) Neurotrophin releasing single and multiple lumen nerve conduits. *J Control Release* 104(3):433–446
- Yang Y, Ding F, Wu J, Hu W, Liu W, Liu J, Gu X (2007) Development and evaluation of silk fibroin-based nerve grafts used for peripheral nerve regeneration. *Biomaterials* 28(36):5526–5535
- Yao L, Billiar KL, Windebank AJ, Pandit A (2010) Multichanneled collagen conduits for peripheral nerve regeneration: design, fabrication, and characterization. *Tissue Eng Part C Methods* 16(6):1585–1596
- Young RC, Wiberg M, Terenghi G (2002) Poly-3-hydroxybutyrate (PHB): a resorbable conduit for long-gap repair in peripheral nerves. *Br J Plast Surg* 55(3):235–240
- Yu W, Zhao W, Zhu C, Zhang X, Ye D, Zhang W, Zhou Y, Jiang X, Zhang Z (2011) Sciatic nerve regeneration in rats by a promising electrospun collagen/poly(ϵ -caprolactone) nerve conduit with tailored degradation rate. *BMC Neurosci* 12:68–68
- Zhao K, Deng Y, Chun Chen J, Chen G-Q (2003) Polyhydroxyalkanoate (PHA) scaffolds with good mechanical properties and biocompatibility. *Biomaterials* 24(6):1041–1045



Fibrin in Nerve Tissue Engineering

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Abstract

This chapter will focus on the use of fibrin for peripheral nerve repair and regeneration. Important basic aspects of fibrinogen and fibrin will be summarized to elucidate the role of fibrin in physiologic processes such as hemostasis and wound healing. The pivotal role of fibrin in nerve regeneration will be discussed in the following section, as current approaches to enhance peripheral nerve regeneration are based on our momentary understanding of the physiological process of neuronal regeneration. The main focus of this work will be on the plethora of applications, which have been developed to take advantage of and modify fibrin's intrinsic biological properties in peripheral nerve repair and regeneration. This chapter will shed light on its use as adhesive for the repair of severed nerves and to stabilize neurorrhaphy sites in order to protect sutured nerves. Nerve conduits made from fibrin will also be discussed, as well as the multitude of applications in which fibrin serves as a carrier for neurotrophic factors and other molecules to enhance nerve regeneration in vivo and in vitro. The following pages are thought to meet the needs and expectations of both clinicians, for example, plastic surgeons, orthopedic surgeons, and neurosurgeons, as well as of basic researchers, for example, biologists. Advantageous features of fibrin from a physician's point of view will be highlighted in addition to chemical and molecular aspects which might be of special interest for basic researchers in the laboratory. In summary, we aim to give a comprehensive overview on fibrin and its application from "bench to bedside" in translational research.

1 Introduction

Nerve injuries can result in either loss of function of motor, autonomic, or sensory nerves and functional recovery is often poor and incomplete (Menorca et al. 2013; Sulaiman and Gordon 2013). In contrast to the vast amount of experimental studies on peripheral nerve injuries and repair, relatively few data were published regarding the epidemiologic and socioeconomic background. Noble and colleagues published their findings on the incidence of upper and lower extremity nerve injuries more than 20 years ago (Noble et al. 1998). They reported that between 2% and 3% of all trauma patients admitted to a regional trauma center were subjected to traumatic

peripheral nerve injuries (PNIs). According to *ResearchGate*, this paper has been cited more than 490 times until October 2019, emphasizing the relatively low output of current data regarding the burden of peripheral nerve injuries in a socioeconomic and epidemiologic context. This burden is not only related to the individual course of disease, but also to costs and sequelae of such injuries for the society. In Germany, about 3.3% of patients with upper extremity trauma also suffer peripheral nerve injuries, whereas 1.8% of patients with lower extremity trauma are affected (Huckhagel et al. 2018a, b). In the USA, the mean care charge of peripheral nerve injury of the upper extremity is estimated to be around \$47,000, while it is estimated that around 44 out of one million people of the US population suffer from such an injury annually. Although the overall incidence of upper extremity PNIs has decreased in recent years, costs are increasing with an annual growth rate of about 9.6% (Karsy et al. 2019). A recent study featuring 250 patients with upper extremity nerve injuries estimated that 16% of patients required rehabilitation with a mean stay duration of 41 days and average costs of more than \$6300. Out of the 250 patients, 30% suffered from long-term sequelae and disabilities from the injury, requiring a mean yearly disability pension of more than \$3800 (Bergmeister et al. 2020). In regard to PNIs of the lower extremity, 13.3 out of one million people are affected every year. Estimated annual costs are around \$64,000 with a growth rate of 8.8% per year (Foster et al. 2019). In both cases, patients are younger and more likely male compared to their counterparts without nerve injury. An estimate of 25% of this population is still unable to return to their workplace 18 months after injury (Ewald and Beckmann-Fries 2017; Davis et al. 2011). Personal and socioeconomic consequences of PNIs depend on the severity of the lesion, and complex injuries like brachial plexus injuries and thoracic outlet syndrome are more likely associated with disastrous psychosocial effects (Wojtkiewicz et al. 2015). Especially patients with brachial plexus injuries are very likely to suffer anxiety and depression in about 40% of cases (Yannascoli et al. 2018) and about one third of the patients admit to having suicidal thoughts (Landers et al. 2018). Development of pain conditions after such injuries is a common problem and especially the socioeconomic consequences of neuropathic pain after traumatic nerve injuries are immense, ranging between \$25,000 and \$30,000 per case and year (Davis and Curtin 2016; Schaefer et al. 2014).

2 What Is Fibrin?

The protein that would later become known as fibrin was originally described by Italian anatomist Marcello Malpighi in 1666 as “polypus cordis.” He assumed appearance of this clot to be related to changes of the blood’s “proper fluid nature” (Forrester 1995). In 1788, Antoine Fourcroy attributed the name “fibrin,” but it was not until 1838 that Berzelius defined the term “protein.” Nine years later, German pathologist Rudolf Virchow discovered the protein’s active form to be derived from a precursor, which he named “fibrinogen” (Hartley 1951; Costa-Filho et al. 2016). In the 1870s, Schmidt elucidated the enzymatic process of this conversion and 7 years

later Hammarsten pioneered purification of fibrinogen (Pieters and Wolberg 2019). Fibrinogen is a hexameric homodimer, consisting of three compounds of two identical polypeptide chains (Mosesson 2005; Mosesson et al. 2001). The genes for these chains are encoded by the FGA, FGB, and FGG genes on chromosome 4p (Espitia Jaimes et al. 2018). Besides a basal, constitutive expression in hepatocytes, fibrinogen expression is also inducible and regulated transcriptionally and translationally (Fish and Neerman-Arbez 2012; Cronje et al. 2017). After assembling of fibrinogen in hepatocytes, it is secreted into the blood. Fibrinogen (factor I) is enzymatically proteolyzed by thrombin to fibrin, one of the key players in hemostasis. Mechanically, the protein exhibits viscoelastic properties in combination with great elasticity. Besides formation of the fibrin network by fiber branching, the protein can also form a sheet-like layer with antimicrobial properties (O'Brien 3rd et al. 2008; Macrae et al. 2018). Fibrin protects blood clots from lysis and disruption, significantly increasing their mechanical properties (Alshehri et al. 2015). Integrin-mediated anchoring of thrombi to endothelial cells by fibrin also contributes to clot stability (Suehiro et al. 1997; Bevilacqua 1993). On the other hand, after binding to $\alpha_{IIb}\beta_3$ -receptors on platelets, it promotes thrombocyte aggregation and contraction of the blood clot (O'Toole et al. 1990). Fibrin can also bind to the $\alpha_M\beta_{II}$ integrin expressed on leukocytes to modulate inflammatory processes (Trezzini et al. 1988; Altieri et al. 1990) and affect blood rheology by interacting with erythrocytes (Aleman et al. 2014; Walton et al. 2015). Due to its key role in hemostasis and wound healing, fibrinogen is used in a wide variety of applications in human patients, taking advantage of its remarkable mechanical properties (Saltz et al. 1991; Fattahi et al. 2004; Noori et al. 2017; Pieters and Wolberg 2019).

Use of fibrin was pioneered by Bergel in 1909, who used a powder of it for hemostasis (Bergel 1909). However, while fibrin was approved and broadly used in Europe, its use was prohibited in the USA since the FDA did not approve homologous fibrin sealant due to the risk of transmission of infectious diseases such as Hepatitis or HIV. A shift of paradigms occurred in 1983 and 1985, respectively, with the work of Gestring and Dresdale, who combined thrombin and fresh frozen plasma from pooled donors previously tested negative for infectious diseases to manufacture fibrin glue. This made approval by the FDA possible in 1998 (Gestring and Lerner 1983; Dresdale et al. 1985; Spotnitz and Prabhu 2005; Spotnitz 2010). Today, the only fibrin sealant class approved by the FDA consists of human blood plasma pooled fibrinogen and thrombin (Spotnitz 2014). Out of all alternative sealants based on and derived from fibrin, the authors of this chapter want to mention at least the two, which they consider to be the most frequently used ones in experimental studies. This is on the one hand heterologous fibrin sealant from snake venom (HFS), which has been reported to produce equal results to conventional fibrin sealant in several studies (Iuan et al. 1995; Barbizan et al. 2013; Buchaim et al. 2015; Vieira et al. 2016; Perussi Biscola et al. 2016; Vidigal de Castro et al. 2016; Rosso et al. 2017; Biscola et al. 2017). On the other hand, platelet-rich fibrin (PRF) was investigated as an alternative to conventional fibrin sealant in several studies with varying results (Yu et al. 2018). In case of nerve coaptation, there are anecdotal reports of good results in human patients (Khojasteh et al. 2016), while animal

studies show different findings, ranging from equal results compared to autologous nerve grafts (Roth et al. 2017) over ambivalent findings (Torul et al. 2018; Bayram et al. 2018) to reports of inferiority of the method (Senses et al. 2016). Some authors even advocated against the use of PRF for peripheral nerve repair after resection of a malignant tumor due to an assumed carcinogenic effect (Spartalis et al. 2018).

2.1 Fibrin, the End-Product of Blood Coagulation

Coagulation, the formation of a blood clot, is an essential process for wound closure and thus a highly conserved mechanism ubiquitous in all vertebrates (Doolittle et al. 1997). In general, blood clotting is mediated by a complex interplay between blood cells, plasma proteins (coagulation factors), and other circulating factors released from surrounding tissues such as the endothelial lining inside a blood vessel or the subendothelial space. The blood coagulation cascade involves a spatiotemporally regulated series of enzymatic reactions, whereby soluble proteins are converted into an insoluble fibrous structure, the blood clot (thrombus). This process is paramount to hemostasis and ultimately results in the formation of a platelet plug accompanied with the deposition of fibrin, a semi-rigid hydrogel network that forms the structural basis of the blood clot (Davie et al. 1991).

Coagulation is triggered by injury to a blood vessel, resulting in damage to its endothelium. Exposure of blood to the subendothelial space (and the injury itself) initiates a cascade of enzymatic reactions involving the proteolytic activation of coagulation factors which otherwise circulate as inactive zymogens. These factors are classified by the roman numerals F (for factor) I–XIII, with the lowercase appendix “a” indicating an active form. Most coagulation factors are serine proteases, but a few exceptions exist. For example, FV and FVIII are glycoproteins, and FXIII is a transglutaminase. The traditional “cascade” or “waterfall” models of blood clotting, identifying coagulation as a series of enzymatic reactions, were originally described in the 1960s (Davie and Ratnoff 1964; Macfarlane 1964), and the last step of this cascade is the conversion of fibrinogen (FI) to fibrin (F1a) by the activated serine protease thrombin (FIIa). This leads to the formation of a polymeric fibrin network which is subsequently stabilized by FXIIIa-mediated crosslinking of fibrin polymers to form a stable fibrin clot.

Hemostasis is generally achieved by two simultaneously occurring processes, primary and secondary hemostasis. Primary hemostasis is initiated by the adhesion, activation, and aggregation of platelets, leading to the formation of a platelet plug at the site of vessel injury (Clemetson 2012). Compared to the rather antithrombotic endothelium (which is compromised upon injury), the subendothelial space is a very different environment, rich in extracellular matrix (ECM) proteins (mostly collagen, laminin, fibronectin, and vitronectin) and, importantly, collagen-bound von Willebrand Factor (vWF). Exposure to this highly thrombogenic microenvironment tethers platelets to the subendothelium through interaction between the platelet glycoprotein complex Ib (GP1b) receptor and vWF (Heemskerk et al. 2002). Binding of vWF activates platelets to secrete a variety of factors which further enhance

platelet activation through auto- and paracrine feedback loops. This is accompanied by a change in platelet surface receptor expression, as activated platelets induce expression of a set of integrins (including integrin $\alpha_{IIb}\beta_3$), enabling them to bind to ECM proteins as well as to fibrinogen (Bennett 2005; Charo et al. 1987). As fibrinogen is a divalent ligand for $\alpha_{IIb}\beta_3$ integrins, two platelets can bind to the same fibrinogen monomer, allowing for platelet aggregation. Secondary hemostasis involves the aforementioned thrombin-mediated conversion of fibrinogen to fibrin, whose subsequent addition to the platelet plug provides the structural basis of the blood clot. Traditionally, the coagulation cascade has been classified into the intrinsic and extrinsic pathways which eventually converge into the common pathway at the level of FX activation. Activated FXa, together with its co-factors FVa, phospholipids, and Ca^{2+} , is often referred to as the prothrombinase complex acting upstream of thrombin. FXa-mediated cleavage of prothrombin yields activated thrombin (FIIa) which simultaneously catalyzes fibrin formation and fibrin crosslinking through activation of FXIII (Owens 3rd and Mackman 2010). The peculiarities of the intrinsic and extrinsic coagulation pathways are beyond the scope of this chapter, but several comprehensive reviews on this matter are available for the interested reader (Owens 3rd and Mackman 2010; Gailani and Renne 2007; Lasne et al. 2006; Mackman et al. 2007; Renne and Gailani 2007; Veldman et al. 2003).

Summarizing, the blood coagulation cascade is a highly complex and tightly regulated series of events that involves the orchestrated interplay of diverse signals such as the presence/exposure of specific ECM proteins, blood cells, or circulating coagulation factors, to name a few. It is, however, noteworthy to point out that under physiological conditions, the blood clot is generally not a permanent structure. As unregulated blood clotting will lead to permanent vessel occlusion (thrombosis), the regulation of the delicate balance between the pro- and anticoagulant circuits is of extreme clinical importance (Kalafatis et al. 1997).

2.2 The Central Role of Fibrin in Wound Healing

Wound healing is a highly dynamic process governed by humoral, cellular, and molecular mechanisms. Fibrin is centrally involved in all stages of wound healing, from hemostasis and modulation of inflammatory processes to re-epithelialization, fibroplasia, (neo)angiogenesis, and remodeling (Clark 2001; Laurens et al. 2006). Apart from its primary role, hemostasis, it also acts as a provisional matrix for the many cell types involved in tissue repair. Notably, fibrin not only provides structural support for cellular infiltration, making it one of the most important ECM proteins in the wound area, but also performs several regulatory functions on the cellular and molecular level (Clark 2001; Laurens et al. 2006; Reinke and Sorg 2012). Prominent examples of such signaling functions include: (i) the direct modulation of the inflammatory response by coagulation factors, for example, FXIIa (Hageman factor), which stimulates the secretion of potent vasoactive agents like bradykinin (Muller-Esterl 1989) and also activates the complement system (Ghebrehiwet et al.

1981); (ii) the integrin-mediated modulation of immune cell phenotype, function, and secretome upon binding to fibrin (Altieri et al. 1990; Juliano and Haskill 1993; Trezzini et al. 1991); (iii) the fibrin-based (in combination with fibronectin and vitronectin) regulation of fibroblast (Greiling and Clark 1997) and endothelial cell (Clark et al. 1996) influx during the formation of granulation tissue; (iv) the direct induction of wound angiogenesis (Lasne et al. 2006; Dvorak et al. 1987; Jakob et al. 1982); (v) the binding of growth factors (GFs) and cytokines involved in wound repair and tissue remodeling, such as vascular endothelial growth factor (VEGF) (Sahni and Francis 2000), basic fibroblast growth factor (FGF2, bFGF) (Sahni et al. 2003), or interleukin 1 β (IL1) (Sahni et al. 2004); or (vi) the chemotactic modulation of cellular behavior by fibrinolytic processes and fibrin degradation products (FDPs) (Jennewein et al. 2011; Wojtecka-Lukasik and Maslinski 1992).

The physicochemical versatility of fibrin, in combination with its intrinsic biological signaling capabilities, makes the blood clot an extremely efficient and elegant system to achieve hemostasis while simultaneously promoting wound healing and repair. Nevertheless, once the blood clot is present in the wound, the cells must deal with it (Clark 2001), which involves spatiotemporal interactions between a variety of invading cell types and fibrin. Although other ECM proteins contribute to cell adhesion (by also interacting with fibrin), the importance of instructive signals stemming from the fibrin matrix itself as a prerequisite for proper wound healing has been unambiguously demonstrated.

2.3 From Wound Healing to Nerve Regeneration

Fibrinogen and fibrin, respectively, also play an important role in peripheral nerve injuries and regeneration (Akassoglou and Strickland 2002; Belkas et al. 2004). In the 1980s, Lundborg and his group emphasized the importance of the distal nerve stump for nerve regeneration after segmental injury, although fibrin's pivotal role was not the focus of their extensive work (Lundborg et al. 1982a, b, c, d; Lundborg 1982a, b). Williams and colleagues extensively studied the spatiotemporal process of peripheral nerve regeneration within a silicon chamber (Williams et al. 1983). After a nerve is cut, a clot mainly consisting of fibrin and fibronectin is formed to connect the two severed nerve ends (Williams and Varon 1985; Williams 1987; Le Beau et al. 1988; Liu 1992; Hoffman-Kim et al. 2010). Some authors also refer to this clot as a "cable" as it can cover distances of several centimeters depending on the length of the nerve injury. Interestingly, the clot is also present when axons are prevented from entering the compartment between two nerve stumps and its size is dependent on the degree of vascularization of the nerve stumps (Zhao et al. 1992; Williams et al. 1993). Following formation of the fibrin cable, it is infiltrated by perineurial cells, endothelial cells, fibroblasts, and, most importantly, Schwann cells (Kaplan et al. 2015). After migration, Schwann cells form the bands of Büngner, which are critically important for nerve regeneration by providing a guiding structure for regenerating axons (von Büngner 1891). By inducing phosphorylation of extracellular signal regulated kinase (ERK) 1/2 and increasing expression of the low affinity

nerve growth factor (NGF) receptor p75 in Schwann cells, fibrin maintains them in a nonmyelinating state while also inhibiting their further migration (Akassoglou et al. 2002, 2003). As the fibrin cable acts as a scaffold for proregenerative cells, its degradation time is crucial for nerve regeneration. In humans, the degradation is estimated to be completed within 4 weeks, defining the maximum gap length between two nerve stumps of approximately 4 cm (Kaplan et al. 2015). Interestingly, degradation occurs much faster in rats and the fibrin cable is broken down after 14 days, resulting in a maximum gap size of 1.5 cm even though the speed of axonal regeneration is much faster in rodents than in humans (Kalbermatten et al. 2008; Pettersson et al. 2010). Degradation of the fibrin cable is dependent on proteases and their activation by tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), since mice deficient for these proteins display strongly limited functional recovery following nerve injury (Siconolfi and Seeds 2001a, b, 2003). The fibrin cable's interaction with surface textures in its proximity is another crucial factor for nerve regeneration. Whereas smooth surfaces promote formation of discrete, free-floating nerve cables, rough surfaces induce formation of loose connective tissue constructs with only minimal neuronal structures contained within (Aebischer et al. 1990).

3 Mechanisms of Fibrin Formation and Degradation

Fibrin formation (fibrinogenesis) and degradation (fibrinolysis) are fundamental mechanisms for coagulation and tissue repair. The efficiency of wound healing crucially relies on the initial plugging of a vascular injury followed by consecutive tissue remodeling and timely degradation of the blood clot to ensure tissue integrity. In the healthy human body, the dynamic processes of fibrinogenesis and -lysis are in a delicate balance, the hemostatic balance (Astrup 1958), malfunctions of which can lead to excessive clotting (thrombosis) or bleeding (hemorrhage) and may severely impair tissue remodeling and repair (Sidelmann et al. 2000).

3.1 Fibrinogenesis and Fibrin Network Formation

3.1.1 Fibrinogen, Thrombin, and FXIII

Within the common pathway of coagulation, fibrinogen, thrombin, and FXIII are the key players acting in the formation of a fibrin clot. Fibrinogen (FI), the monomeric building block of fibrin, is a 340 kDa plasma glycoprotein primarily produced by hepatocytes (and, to some extent, platelets) which circulates freely in the plasma at a concentration around 1.5–4 mg/mL (Sierra 1993). Fibrinogen is a dimeric molecule consisting of two sets of polypeptides, each termed A α , B β , and γ , which are linked by a total of 29 disulfide bonds (Weisel 2005). These polypeptide chains form a 45 nm long elongated structure that comprises two outer D domains, formed by the B β and γ chain C-termini (β_C and γ_C), and a central E domain harboring the N-termini of all 6 polypeptide chains (Fig. 1) (Mosesson 2005; Undas and Ariens

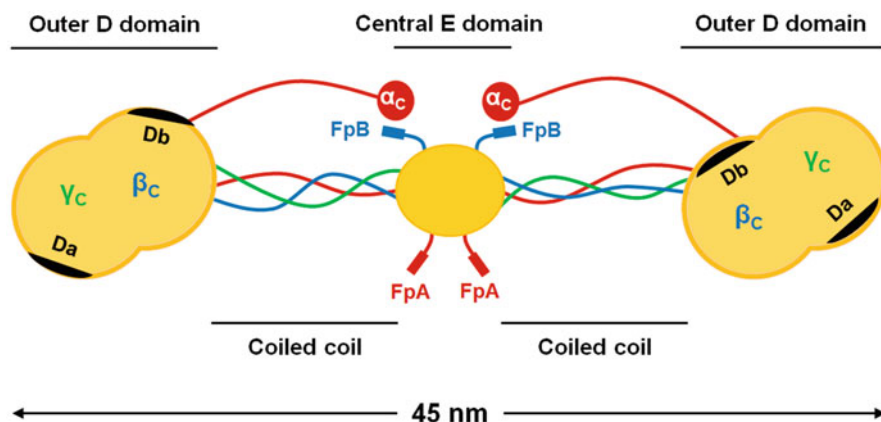


Fig. 1 The fibrinogen monomer with its most important functional domains. (Adapted from Mosesson 2005)

2011). In contrast to β_C and γ_C , the globular A α C-termini (α_C) are located in proximity to the E domain (Tsurupa et al. 2009; Veklich et al. 1993). Two α -helical coiled-coil segments connect the outer D domains on either side of the fibrinogen monomer with the E domain, where the polypeptide chains are joined together by additional disulfide bridges (Blomback et al. 1976; Hoeprich Jr. and Doolittle 1983). Importantly, each A α and B β chain contains an N-terminal fibrinopeptide (FpA and FpB, respectively), proteolytic cleavage of which by thrombin initiates fibrinogenesis (Blomback et al. 1978).

The A α chains are made up by 610, the B β chains by 461, and the major γ chain form (γ A) by 411 amino acid residues. A minor γ chain splice variant (γ') containing a unique anionic 20 amino acid sequence also exists, accounting for approximately 8% of the total fibrinogen γ chain population (Chung and Davie 1984). Cells can interact with fibrin(ogen) via integrin receptor-mediated binding to two distinctive primary recognition motifs, arg-gly-asp-x (RGD) on the A α chains and ala-gly-asp-val (AGDV) on the γ chains (Cheresh et al. 1989; Hettasch et al. 1992). In addition, the D domains contain multiple Ca²⁺ binding sites on the B β and γ chains (Weisel 2005) which take part in the modulation of fibrinogenesis and -lysis through regulation of Ca²⁺ availability, an important co-factor for thrombin and FXIII (Mihalyi 1988; Odrliin et al. 1996a).

Like fibrinogen, the serine protease thrombin (39 kDa) is produced by hepatocytes and circulates as the inactive zymogen prothrombin (FII) at plasma concentrations around 0.15 mg/mL (Sierra 1993). During the blood coagulation cascade, thrombin is activated by FXa which cleaves prothrombin in a Ca^{2+} -dependent manner. Activated thrombin (FIIa) then cleaves FpA and FpB on the $\text{A}\alpha$ and $\text{B}\beta$ chains to initiate fibrin polymerization into protofibrils. At the same time, thrombin proteolytically activates FXIII which subsequently crosslinks the nascent fibrin polymer by introduction of inter- and intramolecular amide bonds between the α

and γ or between two α or γ chains of neighboring fibrin monomers (Weisel and Litvinov 2013).

The transglutaminase FXIII (326 kDa) circulates as an inactive heterotetrameric zymogen consisting of two catalytic A subunits (FXIII-A) and two carrier B subunits (FXIII-B) (Schwartz et al. 1973). The FXIII subunits are produced at different sites in the human body, with FXIII-A being produced by cells in the bone marrow and FXIII-B by hepatocytes. The two different subunits form a noncovalently bound complex in circulation (termed A_2B_2) and physiological plasma concentrations can reach up to 28 $\mu\text{g/mL}$ (Bagoly et al. 2012). The majority of FXIII in circulation is actually bound to fibrinogen (Moaddel et al. 2000) which not only acts as a carrier for FXIII but also regulates its activity (Lorand 2001). The thrombin-mediated activation of FXIII occurs in association with fibrinogen itself, evident by the notion that FXIII activation is significantly accelerated by the presence of fibrinogen and fibrin (Janus et al. 1983; Lewis et al. 1985). This process is co-regulated by Ca^{2+} which controls FXIII activation not only by regulating thrombin activation (which in turn activates FXIII) but also by acting as a co-factor for thrombin-mediated FXIII activation as well (Lorand and Konishi 1964).

3.1.2 Fibrinogenesis

The formation of a stable fibrin network involves a series of consecutive enzymatic and nonenzymatic reactions, each of which will determine the final ultrastructure and overall physicochemical properties of the resulting clot. Dysfunctions in fibrinogenesis and -lysis play pivotal roles in various pathologies, including heart attacks, ischemic stroke, cancer, trauma, hereditary, or acquired coagulopathies or thrombocytopathies (Undas and Ariens 2011). The series of macromolecular assembly steps leading to the formation of a stable polymeric fibrin network can be divided into the following mechanisms (Fig. 2) (Weisel and Litvinov 2013):

1. Cleavage of fibrinopeptides
2. Interactions between polymerization sites and complementary binding pockets (“knob-hole” interactions)
3. Fibrin oligomer and protofibril formation
4. Lateral aggregation and fibrin fiber formation
5. Fibrin crosslinking by FXIIIa (initiated at the time of protofibril formation)
6. Fiber branching and network (clot) formation

As mentioned above, fibrinogenesis is initiated by thrombin-mediated cleavage of the fibrinopeptides FpA and FpB from the N-termini of $A\alpha$ and $B\beta$ chains, converting the fibrinogen monomer into a fibrin monomer $(\alpha\beta\gamma)_2$ (Blomback et al. 1978). This elicits structural changes in the central E domain and exposes two N-terminal polymerization sites, E_A and E_B (“knobs” A and B), in the α and β chains, respectively (Laudano and Doolittle 1978; Pandya et al. 1985; Shainoff and Dardik 1983). Each E_A and E_B site recognizes a complementary binding pocket, Da and Db (“hole” a and b), in the D domain of adjacent fibrin monomers (Everse et al. 1998). Initially, these $E_A\text{:Da}$ and $E_B\text{:Db}$ interactions between the E and D domains

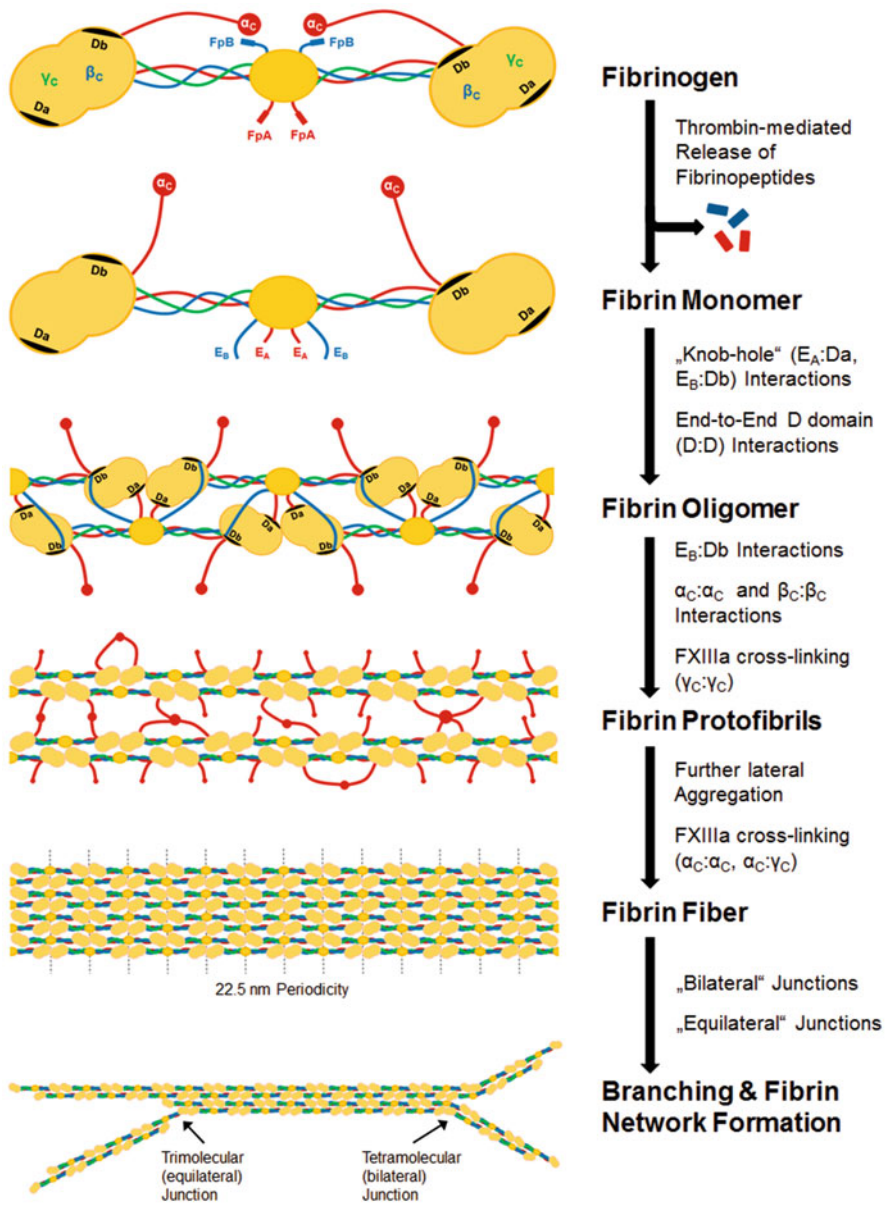


Fig. 2 The thrombin-mediated initiation of fibrinogenesis. (Adapted from Weisel and Litvinov 2013)

induce the aggregation of two fibrin monomers in a half-staggered manner (Fig. 2). Subsequent longitudinal addition of fibrin monomers to the half-staggered fibrin dimer generates a double-stranded fibrin oligomer, the twisted protofibril, in which

monomers are aligned in an end-to-middle overlapping domain arrangement (Medved et al. 1990). E_A:Da interactions are the driving force behind protofibril formation and have been found to be about six-fold stronger than E_B:Db interactions (Litvinov et al. 2005), which seem to play a more central role in lateral fiber aggregation. In addition, interactions between two neighboring D domains, so-called D:D interactions, stabilize the end-to-end junctions between the fibrin monomers (Spraggon et al. 1997). Although D:D interactions are comparably weak, they are nonetheless necessary for proper end-to-end alignment of fibrin monomers in the assembling protofibril (Mosesson et al. 1995).

The interactions governing lateral aggregation of protofibrils to yield a fibrin fiber are weaker than the E_A:Da interactions driving protofibril formation. Therefore, the protofibril must reach a sufficient length, at least 500 nm or 20–25 half-staggered fibrin monomers (Chernysh et al. 2011), before lateral aggregation through cumulative intermolecular forces can occur (Hantgan et al. 1980). Several structures on the fibrin monomer are involved in this process, such as E_B:Db interactions (Weisel and Litvinov 2013), α_C regions (Litvinov et al. 2007), β_C regions (Yang et al. 2000), or the coiled coils between the E and D domains (Okumura et al. 2006). For the sake of brevity, only the most important factors in lateral aggregation are mentioned here, as the precise mechanisms have been comprehensively reviewed in detail elsewhere (Weisel and Litvinov 2013). Due to lateral aggregation, fibrin fibers are paracrystalline structures, meaning that the monomers are regularly arranged longitudinally but less ordered across the fiber. The intermolecular interactions stabilizing the fibrils in the fiber are specific, so that a fiber displays repeats of half the length of a fibrin monomer (22.5 nm), which can be visualized as a distinctive band pattern by electron microscopy or X-ray diffraction (Cohen et al. 1963; Stryer et al. 1963).

Upon fibrin polymerization, the transglutaminase FXIII is activated by the concerted actions of thrombin and Ca²⁺. FXIIIa crosslinks the nascent fibrin network by introducing covalent bonds between fibrin monomers, thus dramatically increasing clot strength and resistance to proteolytic degradation (Lorand 2001). This process is essential for hemostasis, as FXIII deficiency is the root cause of severe bleeding disorders (Levy and Greenberg 2013). The correct timing of FXIII activation is crucial, and the key feature of the synchronization of fibrinogenesis with FXIII activation is the dual role of thrombin in initiating both processes. FXIII activation is greatly enhanced by the presence of fibrin and involves the formation of a tight ternary complex between FXIII, thrombin, and fibrin (Janus et al. 1983; Lewis et al. 1985). FXIIIa is activated in a two-step process and catalyzes the formation of reciprocal intermolecular ϵ -(γ -glutamyl)lysine bonds between the side chains of ϵ -lysine (donor) and γ -glutamine (acceptor) residues (reviewed by Lorand 2001). These covalent bonds occur between two adjacent γ chains (Doolittle et al. 1971), two adjacent α chains (Matsuka et al. 1996), or between α and γ chains (Siebenlist and Mosesson 1996). In addition, FXIIIa can also crosslink other substrates to fibrin, for example, fibrinolytic agents, GFs, or ECM proteins, and thus adds an extra regulatory layer in the modulation of the hemostatic balance (Bagoly et al. 2012; Ariens et al. 2002).

The final step of fibrinogenesis is the formation of a three-dimensional fibrin network which involves fiber branching (Fig. 2). Evidence from structural studies suggests two mechanisms by which branches in the fibrin network may occur (Mosesson et al. 1993). Bilateral junctions form when a double-stranded protofibril converges laterally with another protofibril to create a four-stranded fibril, resulting in tetramolecular branch points (Hantgan et al. 1980; Hermans and McDonagh 1982; Weisel and Nagaswami 1992). Equilateral junctions arise from convergent interactions among three fibrin molecules that give rise to three double-stranded protofibrils, generating trimolecular branch points (Fogelson and Keener 2010; Mosesson et al. 1989). Interestingly, lateral aggregation and fiber branching seem to compete, as the number of branch points and fiber diameter are inversely proportional (Ryan et al. 1999). In addition, fiber branching is partially regulated by the kinetics of fibrinogenesis, with less branched and more porous networks when thrombin activity is high (Blomback et al. 1994). Thus, conditions that favor lateral aggregation yield fibrin clots with thick fibers and fewer branch points (coarse networks) while conditions that inhibit lateral aggregation yield clots with thinner fibers and many branch points (fine networks).

3.2 Fibrinolysis

As already mentioned in the course of this chapter, the blood clot is not a permanent structure under physiological conditions. Once it has served its purpose, it needs to be degraded to prevent permanent occlusion of the blood vessel and to not interfere with tissue remodeling. To preserve the hemostatic balance, the coagulation system is counteracted by fibrinolytic processes, and fibrin plays a central role in this balance as it is the primary product of coagulation as well as the ultimate substrate for fibrinolysis. Similar to the mechanisms at play in fibrinogenesis and blood coagulation, the fibrinolytic cascades are surface-related and involve a plethora of fibrinolytic enzymes (fibrinolysins), their receptors and regulators (Cesarman-Maus and Hajjar 2005).

3.2.1 Regulation of the Fibrinolytic System

In vivo fibrinolysis is mainly mediated by the plasminogen (PLG) pathway. Like many factors involved in hemostasis, PLG (92 kDa), the inactive zymogen of the main fibrinolysin plasmin, is primarily produced by hepatocytes (Raum et al. 1980). PLG contains five homologous 80 amino acid long triple loop structures called “kringles” (Forsgren et al. 1987), with the first (K1) and fourth (K4) kringle acting as binding domains for the interaction with fibrin, PLG regulators, and cell surface receptors (Plow et al. 1991). Fibrin, the very substrate for fibrinolysis itself, plays an integral role in the activation of PLG as it binds PLG and some of its regulators, thereby facilitating interactions between them (Medved and Nieuwenhuizen 2003). Binding of PLG to fibrin via its kringle domains induces a series of conformational changes in PLG which expose otherwise hidden cleavage (activation) sites for PLG activators (Law et al. 2012; Longstaff and Kolev 2015). The two most prominent

activators of PLG are tPA and uPA, serine proteases that circulate as single chain zymogens (sc-tPA and sc-uPA, respectively). The 72 kDa glycoprotein tPA also contains two kringle domains, homologous to those found in PLG, for fibrin binding (Cesarman-Maus and Hajjar 2005) and the co-localization of tPA, PLG, and fibrin is central for tPA regulation. In the absence of fibrin, tPA is a weak activator of PLG; however, its activity increases 10^2 - to 10^3 -fold in the presence of fibrin (Hoylaerts et al. 1982). In this respect, the formation of localized ternary complexes between tPA, PLG and fibrin not only enables tPA-mediated activation of PLG to yield plasmin, but also elegantly restricts fibrinolysis to the fibrin clot. Similar to tPA, the 54 kDa glycoprotein uPA (also termed prourokinase) also contains a PLG-like kringle domain (Kasai et al. 1985). However, in contrast to tPA, uPA can efficiently activate PLG in both the presence and absence of fibrin, but generally has much lower affinity for fibrin than tPA (Gurewich et al. 1984; Lijnen et al. 1986). Thus, uPA has been linked with cell-mediated fibrinolysis, exerted in combination with a specific uPA-receptor, uPAR (or CD87) (Myohanen and Vaheri 2004). Notably, both sc-tPA and sc-uPA can be cleaved by plasmin as part of a positive feedback loop, transforming the single-chain variants into more active, disulfide-bridged two-chain polypeptides (tc-tPA and tc-uPA, respectively) (Cesarman-Maus and Hajjar 2005; Tate et al. 1987).

Antifibrinolytic mechanisms are equally important for the preservation of the hemostatic balance as fibrinolytic ones. These mechanisms are required to prevent unregulated systemic fibrinolysis and excessive degradation of plasma proteins. The three major ways to negatively regulate fibrinolysis are (i) inhibition of plasmin, (ii) inhibition of PLG activators such as tPA and uPA, and (iii) removal or blocking of the binding sites for PLG/plasmin, tPA, and uPA. The vast majority of fibrinolysis inhibitors belong to a family of serine protease inhibitors known as serpins (Travis and Salvesen 1983). Among the circulating serpins, the three most important fibrinolysis inhibitors are plasminogen activator inhibitor-1 (PAI-1), plasminogen activator inhibitor-2 (PAI-2), and $\alpha 2$ -plasmin inhibitor ($\alpha 2$ -PI or $\alpha 2$ -antiplasmin). Their mode of action generally involves the irreversible formation of high-affinity inhibitory complexes with the active site of the target serine protease which subsequently cleaves the inhibitor, resulting in neutralization of the protease and clearance from circulation (Lawrence 1997). Examples are $\alpha 2$ -PI, the primary inhibitor of plasmin, which forms $\alpha 2$ -PI-plasmin complexes with 1:1 stoichiometry by occupation of plasmin's kringle domains (Wiman et al. 1978), or PAI-1 and PAI-2 which rapidly inactivate tPA and uPA by similar mechanisms of equimolar complexation (Chapin and Hajjar 2015). Alternatively, fibrinolysis can be inhibited by preventing the interaction of PLG/plasmin or its activators with fibrin. A plasma metalloproteinase, the thrombin-activatable fibrinolysis inhibitor (TAFIa), is a potent attenuator of fibrinolysis (Nesheim 2003). TAFI, circulating as inactive zymogen, is activated by thrombin or plasmin (at high concentrations), and its activation is accelerated about 1250-fold in the presence of the endothelial cell membrane protein thrombomodulin (Cesarman-Maus and Hajjar 2005). Once active, TAFIa cleaves lysine residues on fibrin necessary for PLG and tPA binding and the formation of the ternary complex that enables plasmin activation. TAFIa predominantly acts through

modifying the binding affinity of PLG and plasmin for fibrin, and to a lesser extent by affecting tPA binding (Mutch et al. 2003; Silva et al. 2012a). This mode of action is mimicked by clinically used antifibrinolytics such as ϵ -aminocaproic acid (EACA) or tranexamic acid (TEA) which do not decrease the number of lysine residues on fibrin, but competitively inhibit PLG and plasmin binding to fibrin, thus suppressing fibrinolytic activity (Slaughter and Greenberg 1997).

3.2.2 The Fibrinolytic and Nonfibrinolytic Actions of Plasmin

The plasmin-induced degradation of fibrin(ogen) monomers or polymers involves a sequence of proteolytic cleavages which yield a broad array of different FDPs (Fig. 3) (Longstaff and Kolev 2015). Fibrinogen has specific proteolytic cleavage sites for plasmin, with the A α chains presenting an early target for degradation. More specifically, the α_C region is initially cleaved by plasmin, yielding a heterogeneous set of early FDPs (Mosesson et al. 1972), which is followed by the removal of an N-terminal peptide in the B β chains (Koehn et al. 1983). The resulting 250 kDa molecule, called fragment X, actually represents a clottable form of fibrinogen (Fig. 3a). Subsequent cleavage of all three polypeptide chains in the coiled coil connector between the E and D domains of fragment X yields fragment D (100 kDa) and fragment Y (155 kDa) (Kirschbaum and Budzynski 1990; Marder et al. 1969).

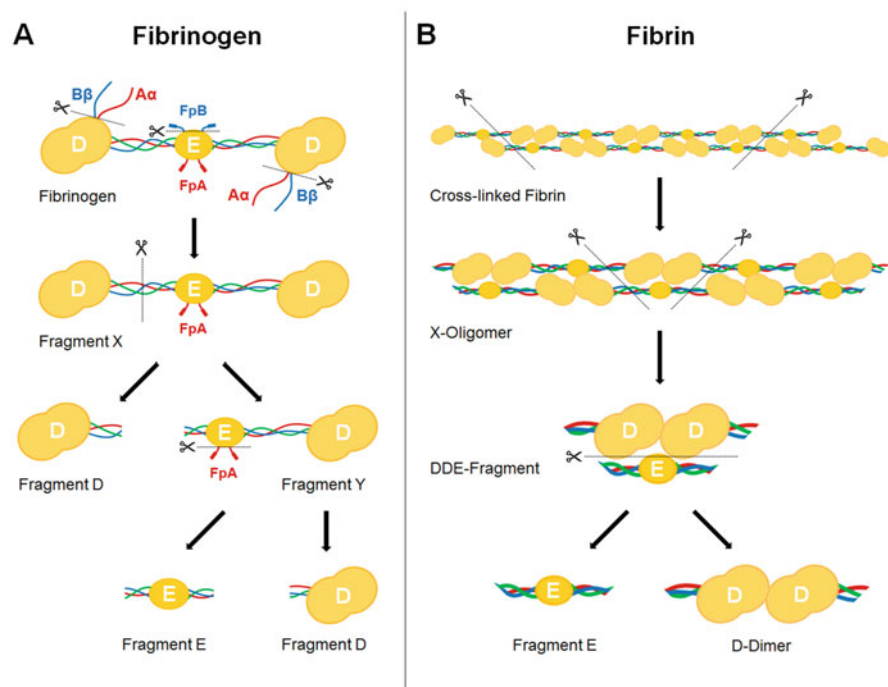


Fig. 3 The plasmin-mediated degradation of fibrinogen and fibrin. (Adapted from Sidelmann et al. 2000; Cesarman-Maus and Hajjar 2005)

A second proteolytic cleavage in the connector region of fragment Y ultimately generates a second fragment D and fragment E, which are the terminal FDPs and basically the major globular domains of fibrinogen (Fowler et al. 1980).

The plasmin-mediated degradation of fibrin monomers as well as that of non-crosslinked fibrin polymers is identical to that of fibrinogen, except for the fact that fibrin polymer degradation yields more complex fragments termed X-oligomers (Fig. 3b) (Gaffney et al. 1988; Graeff and Hafter 1982). As fibrinolysis proceeds, the initially large X-oligomers gradually decrease in size and eventually yield DDE fragments, degradation of which yields the final FDPs fragment E and D-dimer (two covalently bound D domains) (Sidelmann et al. 2000). The quantification of D-dimers in the blood is routinely used in clinics to predict pro- or anticoagulant events. D-dimer assays identify excessive fibrinolysis which may be associated with pathologies such as pulmonary embolisms, deep venous thrombosis, or disseminated intravascular coagulation (Khalafallah et al. 2014; van der Hulle et al. 2013a, b).

It is noteworthy to point out that plasmin also exerts nonfibrinolytic degradative actions. Apart from fibrinogen and fibrin, it may also degrade other ECM proteins. For example, plasmin directly cleaves basement membrane proteins such as thrombospondin, laminin, or fibronectin, while additionally facilitating degradation of collagen, vitronectin, elastin, or aggrecan by activation of matrix metalloproteinases (MMPs) 1 and 3. These nonfibrinolytic proteolytic actions suggest a multidimensional involvement of plasmin in inflammation, tissue remodeling, wound healing, cell invasion, and prohormone activation *in vivo* (reviewed by Cesarman-Maus and Hajjar 2005). Furthermore, plasmin has been found to cleave and inactivate several coagulation factors *in vitro*, indicating that it not only is the primary fibrinolysin, but may also exert direct anticoagulant effects (Hoover-Plow 2010). Among the coagulation factors inhibited by plasmin *in vitro* are FV, FVIII, FIX, and FX (Omar and Mann 1987; Prydzial et al. 1999; Rick and Krizek 1986; Samis et al. 2000), which has sparked speculations about plasmin being a potential master regulator of the intrinsic, extrinsic, and common blood coagulation pathways alike. However, whether the same principles and mechanisms apply to PLG *in vivo* remains elusive, as the rapid thrombolytic activity of plasmin makes it very difficult to detect its possible anticoagulant roles in this setting.

4 Characterization of Fibrin as a Matrix for Nerve Repair

The significant advances in our understanding of blood coagulation and the mechanisms of fibrinogenesis and -lysis over the past decades have led to the commercial development and clinical introduction of fibrin sealants. Fibrin sealants are concentrated plasma-derived two-component systems that elegantly mimic the last stage of coagulation, the thrombin-induced polymerization of fibrinogen (Spotnitz 2010). The first component, the sealer protein, is rich in clottable fibrinogen and FXIII which are solubilized in the presence of an antifibrinolytic (usually aprotinin). The second component, the hardener, contains highly concentrated thrombin solubilized in CaCl_2 . Combination of the two components, usually by dual-syringe applicators,

initiates rapid fibrin polymerization. Although many other nonfibrin surgical sealants are available today (Heher et al. 2018a), the fibrin sealant is still the only system that has Food and Drug Administration (FDA) approval as a hemostat, sealant, and adhesive (Spotnitz 2014). The widespread clinical use of fibrin sealants has also prompted a great deal of research towards a more thorough mechanistic evaluation of fibrin as a scaffold biomaterial for tissue engineering and regenerative medicine (TERM). Even though fibrin sealants are still mainly considered a classic clinical tool, fibrin hydrogels have more recently emerged as a versatile delivery matrix for cells, drugs, gene vectors, and therapeutic biomolecules (Ahmad et al. 2015; Ahmed et al. 2008; Breen et al. 2009; Heher et al. 2018b). This has shifted the focus towards a more thorough evaluation of the fibrin matrix itself, as well as the implications of its biological and physicochemical properties on cellular behavior.

4.1 The Intrinsic Biological Properties of Fibrin

A suitable scaffold material for TERM applications should be biocompatible, biodegradable, nontoxic, and nonimmunogenic and ideally should display mechanical and structural properties appropriate for cell adhesion, migration, proliferation, and differentiation (Yang et al. 2001). Given its natural role in wound healing, fibrin meets many of these requirements as it is a temporary matrix by nature and interacts with a variety of ECM proteins, growth/coagulation factors, and cells. Fibrin has several RGD sites for interaction with a variety of cell types, for example, through integrins $\alpha_{IIb}\beta_3$ (Charo et al. 1987), $\alpha_v\beta_1$ (Suehiro et al. 1997), or $\alpha_v\beta_3$ (Gailit et al. 1997). Alternatively, cell types without natural binding affinity for fibrin can interact with fibrin-bound ECM proteins such as fibronectin or vitronectin (Makogonenko et al. 2002; Podor et al. 2002), thereby (temporarily) binding fibrin indirectly. Apart from its role as a provisional matrix for cells in the blood clot, fibrin also serves as a temporary reservoir for the spatiotemporal release of GFs, chemo- or cytokines. FGF2 (Sahni et al. 2003), VEGF (Sahni and Francis 2000), transforming growth factor β_1 (TGF β_1) (Grainger et al. 1995), platelet-derived growth factor (PDGF) (Dohan et al. 2006), insulin-like growth factor 1 (IGF1) (Dohan et al. 2006), or IL1 β (Sahni et al. 2004) can all be found in the fibrin clot, with some of these factors binding fibrin directly, while others require binding of molecules that bind fibrin.

When used as cell and differentiation matrix, fibrin hydrogels can attain high cell seeding densities and uniform cell distribution while maintaining cell viability and supporting cell adhesion, migration, and proliferation. This is of high relevance for delivery of cells on fibrin matrices *in vivo*, as cells encapsulated in the matrix are protected from the initially harsh conditions after grafting. The presence of (surrounding) fibrin enables the delivered cells to gradually remodel the matrix, differentiate, and/or secrete ECM proteins themselves, a process also supported by invading host cells (Bensaid et al. 2003). This protection and subsequent remodeling is paramount to tissue regeneration which is further supported by fibrin itself providing cues for cell-cell and cell-matrix dependent signaling cascades involved in wound healing. Thus, the delivery of cells within the naturally instructive fibrin

matrix results in improved therapeutic efficiency, as a higher percentage of cells can engraft into damaged tissues compared to local or systemic injections (Christman et al. 2004). Importantly, a big advantage of fibrin as a delivery vehicle in TERM is the fact that fibrin itself as well as some FDPs are pro-angiogenic (Dvorak et al. 1987; Jakob et al. 1982; Knighton et al. 1982), which is key to wound healing.

4.2 Factors Affecting Fibrin Clot Structure and Mechanical Properties

Fibrin is a viscoelastic polymer and its function is greatly influenced by the structural and biochemical properties of the clot (Weisel 2004). One of the disadvantages of natural hydrogels like fibrin is its comparably weak mechanical stiffness, especially for in vivo applications where the fibrin matrix is subjected to much higher fibrinolytic activity than under standardized in vitro conditions. The elastic modulus (Young's modulus or E), a measure for stiffness, and tensile strength are directly proportional to the fibrinogen concentration (Benkherourou et al. 2000; Velada et al. 2002), while thrombin concentrations seem to only have moderate effects on clot strength (Duong et al. 2009). However, thrombin concentrations determine the kinetics of clotting, with direct proportionality of thrombin enzymatic activity and gelation rate. Notably, thrombin-dependent clotting kinetics affect clot microstructure. Low concentrations (<0.1 U/mL) yield coarse fibrin networks with thick, loosely crosslinked fibers, while higher concentrations result in fine networks characterized by thinner, more crosslinked fibers. This allows, to some degree, modulation of clot permeability and porosity, evident by increased fiber diameter in coarse fibrin networks and increased fiber and branching density in fine networks (Collet et al. 2006, 2000). Since Ca^{2+} is an important co-factor of thrombin, fibrin clotting is accelerated at constant thrombin concentrations when Ca^{2+} concentrations are increased (Carr Jr. et al. 1986; Hardy et al. 1983), and vice versa (Godal 1969). Further, Ca^{2+} availability also has effects on clot structure and permeability (Carr Jr. et al. 1986; Okada and Blomback 1983), likely through concerted actions on thrombin and FXIII. Similar to fibrinogen, high FXIII activity greatly increases fibrin stiffness and resistance to lysis (Weisel 2004), emphasized by the severity of bleeding disorders in FXIII-deficient patients (Muszbek et al. 2011). Conversely, inhibition of FXIII activation or FXIII depletion accelerates fibrinolysis in vitro (Jansen et al. 1987) and in vivo (Leidy et al. 1990).

The possibility to modulate the structural and mechanical properties of fibrin is extremely helpful when it is used as scaffold biomaterial for TERM applications (Fig. 4). Cells sensitively react to the physicochemical properties of the fibrin matrix with changes in their adhesion, migration proliferation, and/or differentiation. Obviously, there is no uniform hydrogel composition working similarly for all therapeutic cell types; however, the fact that matrix properties can be modulated allows fine-tuning of the matrix for a given cell type and delivery approach. Concerning the latter, the emergence of nanotechnology-inspired fibrin-based delivery systems such

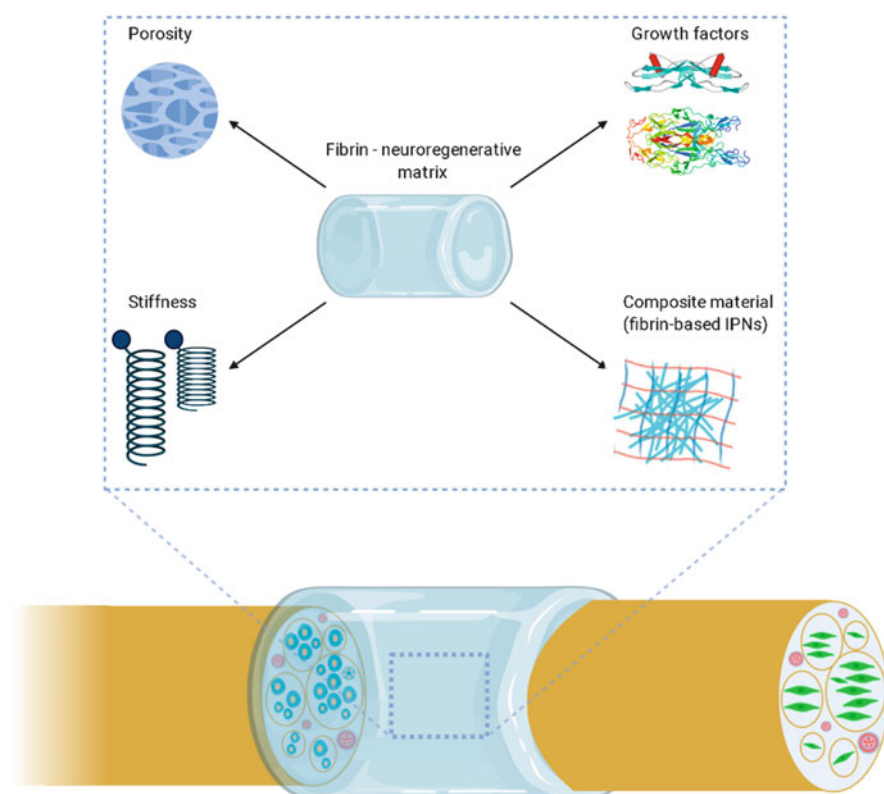


Fig. 4 Structural and biological functionalization approaches of neuroregenerative fibrin matrices

as micro-/nanospheres, nanofibers, microtubes, or nanoparticles might enable controlled therapeutic delivery of defined fibrin micro- or nanostructures without compromising the intrinsic biological properties of fibrin (Rajangam and An 2013).

4.3 Fibrin as a Delivery Matrix for Neuronal and Glial Cells

The implications of fibrin composition on cellular behavior of a variety of therapeutically relevant cell types have been extensively studied. For the sake of brevity, this section will focus on fibrin-based culture and delivery of neuronal (stem) cell types, and examples of matrix functionalization strategies to increase the neurotogenic and neuroprotective properties of fibrin will be given. For the broad range of fibrin-based cell culture and tissue engineering systems, the interested reader is redirected to several comprehensive reviews on these subjects (Ahmad et al. 2015; Ahmed et al. 2008; Heher et al. 2018b; de la Puente and Ludena 2014; Janmey et al. 2009; Whelan et al. 2014).

4.3.1 Factors Affecting Neuronal and Glial Cell Behavior in Fibrin-Based Culture Systems

Among the factors affecting neuronal cell phenotype and function by regulation of fibrin clot structure, the influences of varying fibrinogen and thrombin concentrations have been investigated the most. The nervous system is known to actively react to diverse mechanical stimuli from the microenvironment (Barnes et al. 2017), highlighting the role of substrate stiffness on neuronal function. This can be exploited in in vitro nerve tissue engineering models through the aforementioned fine-tuning of the matrix. For example, a pioneering study by Engler et al. (Engler et al. 2006) found that matrix elasticity governs stem cell lineage specifications. Mesenchymal stromal cells (MSCs) were cultured on varying substrate stiffnesses and very soft matrices which mimic the mechanical properties of the brain directed the cells to differentiate along the neuronal lineage, as opposed to stiff matrices (mimicking bone) which triggered osteogenic differentiation. The importance of target tissue-like matrix stiffness was further investigated by Mooney et al. (Mooney et al. 2010), who reported that soft fibrin matrices made from 5 to 12.5 mg/L fibrinogen not only best supported cell proliferation of a mixture of primary neural cells, but also promoted neuronal rather than glial growth. Similar results were obtained by Bento et al. (Bento et al. 2017), who found a fibrinogen concentration of 6 mg/mL to be optimal for neuronal differentiation of embryonic stem cell (ESC)-derived neural progenitors in fibrin. Interestingly, minor differences in substrate stiffness already elicited biological effects on the cells, as matrices made from 8 or 10 mg/mL fibrinogen were less permissive for neuronal differentiation. To counteract rapid fibrinolysis which limits long-term culture of induced pluripotent stem cell (iPSC)-derived neural progenitors, natural crosslinkers such as genipin have been shown to increase fibrin stability while enhancing neuronal differentiation and neurite outgrowth (Robinson et al. 2017).

Apart from fibrin's mechanical properties, controlled modulation and optimization of its ultrastructure can additionally support nerve regeneration by the provision of positional contact guidance cues (Fig. 4). Especially, methods that induce anisotropic fibrin gels with unidirectional fiber alignment have potential for peripheral nerve repair, as they mimic the regenerative fibrin cable that is subsequently invaded by Schwann cells to form the bands of Büngner. Application of mechanical strain (Heher et al. 2015), magnetic alignment (Dubey et al. 2001), unidirectional freeze-drying (Wu et al. 2010), or electrospinning of hydrogels can generate highly aligned scaffolds, and especially the latter technique has been shown to be a feasible approach with fibrin (McManus et al. 2006, 2007). When seeded with neuronal cells, such electrospun fibrin-based matrices promote cellular alignment accompanied by the formation of bands of Büngner (Schuh et al. 2015), which could greatly enhance their therapeutic efficiency in vivo.

4.3.2 Functionalization Strategies for Neurotrophic Fibrin Matrices

Although fibrin hydrogels generally support survival, growth, and differentiation of neuronal cells (Yu et al. 2020), they are comparably inert in terms of bioactive cues in the matrix other than structural/topological features or cell adhesion motifs. This is

partially due to the fact that, unlike many other GFs involved in tissue repair, neurotrophic GFs do not have natural binding affinity for fibrin. The use of fibrin for topical and sustained delivery of drugs, GFs, and gene vectors has been extensively studied (Breen et al. 2009; Heher et al. 2018b; Whelan et al. 2014), and sustained release of GFs with natural binding affinity for fibrin over days has been achieved by simply encapsulating them into a fibrin matrix. In this scenario, the kinetics of GF release will mainly depend on the rate of fibrinolysis which in turn is dependent on the fibrin composition. Undesirable burst release of GFs without binding affinity for fibrin can be prevented by affinity-based approaches that tether GFs to fibrin via so-called fibrin anchors. A prominent example of this strategy is the incorporation of free heparin into the fibrin clot. Heparin binds fibrin (Odrlić et al. 1996b; Yakovlev et al. 2003) as well as many GFs that do not naturally bind fibrin (Bhang et al. 2007; Jeon et al. 2005, 2008), thus allowing for GF sequestration and sustained release from heparinized fibrin matrices. A major benefit of this approach is that the heparin in the fibrin matrix will also sequester circulating factors in situ (Burgess et al. 1990), thereby increasing their bioavailability at the site of implantation. Furthermore, release kinetics of therapeutic biomolecules from this delivery system can be modulated by adjusting the ratio between heparin and the molecule of interest. While many GFs have high affinity for heparin, a considerable number only have moderate to low affinity, including the neurotrophic GFs neurotrophin-3 (NT-3), NGF- β , and brain-derived neurotrophic factor (BDNF) (Sakiyama-Elbert and Hubbell 2000a). This has prompted research into alternative delivery strategies for these GFs.

The covalent incorporation of GFs into fibrin matrices was a major advancement in the field, allowing for sustained release over longer periods than physical incorporation. Schense and Hubbell (Schense and Hubbell 1999) famously introduced a method for covalent binding of bifunctional peptides to fibrin, elegantly exploiting the FXIIIa crosslinking chemistry during fibrin polymerization. These engineered bi-domain peptides consist of an N-terminal FXIII substrate (derived from α 2-PI) and a C-terminal bioactive domain of choice, for example, a cell binding motif or a GF. Initially, functionalization of fibrin with bifunctional peptides harboring RGD sequences was tested in a neuronal cell culture model, with the outcome that incorporation of the peptide enhanced neurite outgrowth from chicken dorsal root ganglia (Schense and Hubbell 1999). Importantly, the FXIIIa-mediated crosslinking of such peptides into fibrin interfered neither with structural clot integrity nor with the bioactivity of the incorporated growth factor. Similar results on neurite extension were obtained with a heparin-binding variant (based on antithrombin III) of the bi-domain peptide (Sakiyama et al. 1999). FGF2 (which has high affinity for heparin) delivered with this peptide was found to enhance neurite outgrowth from dorsal root ganglia by 100% compared to unmodified fibrin (Sakiyama-Elbert and Hubbell 2000b), highlighting the potency of this approach. The feasibility of the heparin bi-domain peptide approach for sustained release of NT-3, NGF- β , glial cell line-derived neurotrophic factor (GDNF), or PDGF in the context of nerve regeneration has been addressed in several studies, with enhanced neurite extension, improved neuronal progenitor cell survival, and differentiation into functional neurons as

reported outcomes (Johnson et al. 2010; Lee et al. 2003; Taylor et al. 2004; Wood et al. 2009). Sakiyama-Elbert et al. (Sakiyama-Elbert et al. 2001) further refined the bi-domain peptide technique by introducing a fusion protein consisting of an N-terminal FXIII substrate, a plasmin α_2 substrate sequence linker, and a C-terminal GF binding domain. The rationale behind this strategy is that peptide cleavage by endogenous plasmin releases the GF in situ upon cellular demand, with variable release kinetics depending on the local cell activity. The proof of principle was NGF- β delivery, which showed remarkable neurotrophic properties in vitro (Sakiyama-Elbert et al. 2001).

The fibrin-based delivery systems for neurotrophic GF release enhance nerve regeneration in vivo (Fig. 4), as will be discussed in Sect. 5, and have been employed as in vitro neuronal cell culture models to study mechanisms of nerve regeneration (Belanger et al. 2016; Marquardt and Sakiyama-Elbert 2013). Fibrin matrices loaded with GFs have been used as artificial biomimetic niche to foster survival and induce selective commitment of neural progenitor cells prior to transplantation to enrich for therapeutically relevant populations (Chandrababu et al. 2020; Edgar et al. 2017).

4.3.3 Fibrin-Based Interpenetrating Polymer Networks

An alternative approach for functionalization of fibrin, for example, with the bi-domain peptides mentioned earlier, is the addition of other (natural or synthetic) polymers to the fibrin network. This can improve the mechanical properties, delay degradation at the site of transplantation, and incorporate additional bioactive cues into the matrix such as cell adhesion motifs or GF binding sites not otherwise present in fibrin (Fig. 4). In general, interpenetrating polymer networks (IPNs) are composites of two (or more) crosslinked polymeric systems, with at least one of them being polymerized in the presence of the other, but without any covalent bonds between them (Matricardi et al. 2013). A plethora of fibrin-based IPNs, both with natural and synthetic polymers, have been introduced in the last two decades (reviewed by (Brown and Barker 2014)), but only few of them have been characterized in the context of peripheral nerve regeneration. A recent example is a fibrin-collagen IPN, which promoted Schwann cell proliferation and neurite outgrowth in vitro, and led to superior nerve regeneration compared to collagen alone in vivo (Schuh et al. 2018). The IPN approach is also amenable to micropatterning techniques such as electrospinning, as described by Schuh et al. (Schuh et al. 2015), who reported aligned bands of Büngner formation by culturing activated Schwann cells on electrospun fibrin-poly(lactic-co-glycolic-acid) fibers.

5 Fibrin in Nerve Regeneration: In Vivo Applications

5.1 Fibrin Sealant for Sutureless Nerve Repair

“(. . .) a suture material may be discovered of such a composition that it offers little or no obstruction to regeneration, or, on the other hand (. . .) be advantageous because of ease of application. The first gain is biological, the second technical.” – Sir Herbert J. Seddon (1942)

As pointed out by famous Sir Herbert Seddon, use of fibrin sealant instead of sutures for nerve repair was originally sparked by the idea to replace, or at least reduce, the number of sutures for nerve coaptation in order to limit fibrosis and scarring to a minimum (Ngeow 2010). Reduction of these obstacles for axonal regeneration would eventually result in improved axonal regrowth, reinnervation of target organs, and functional recovery (Sarhane et al. 2019).

Experimental use of fibrin sealant to reunite severed nerves was pioneered by zoologist John Young and his disciple and later Noble laureate Peter Medawar in 1940 who used it to repair peripheral nerves of dogs and rabbits (Young and Medawar 1940). Two years later, Sir Herbert J. Seddon, one of the founding fathers of peripheral nerve surgery, pioneered use of fibrin sealant in humans to reunite the median nerve in a patient presenting with a “spaghetti wrist,” an extensive laceration injury of the forearm involving the superficial and deep tendons as well as the median and ulnar nerve (Mafi and Beikpour 2006; Meals and Chang 2018; Seddon and Medawar 1942). Tarlov and colleagues used fibrin sealant for experimental surgery in rabbits, dogs, monkeys, and eventually human patients (Tarlov and Benjamin 1942; Tarlov et al. 1943; Tarlov 1944; Tarlov and Boernstein 1948). In the following years, several other authors published their anecdotal use of this new technique (Sinovskii and Filatov 1950; Metelka et al. 1962; Matsumoto and Chikuba 1966). However, use of fibrin as a natural sealant in nerve repair was not established until the 1970s when Helene Matras published her results of interfascicular nerve grafting in rats, using fibrin sealant (Matras et al. 1972). Four years later, fibrin sealant was successfully used clinically by Kuderna and Matras (Kuderna 1976). Egloff and Narakas used fibrin sealant for nerve repair in a cohort of 56 patients with equal results between fibrin sealant and neurorrhaphy. However, both authors deemed their findings as preliminary and stressed the importance of further studies before drawing a definitive conclusion regarding its efficacy. Additionally, they made use of long grafts to avoid any tension on the coaptation side and primary coaptation was only performed in combination with conventional suturing technique (Egloff and Narakas 1983).

Following this “renaissance” of the use of fibrin to reunite severed nerves, publications regarding this application in animal models increased steadily. Several authors reported equivalent results comparing suture and fibrin sealant repair (Rosso et al. 2017; Becker et al. 1984; Haase et al. 1986; Attar et al. 2012; Pertici et al. 2014; Salmeron-Gonzalez et al. 2018) or deemed fibrin sealant the superior method (Martins et al. 2005a, b; Ornelas et al. 2006a, b; Adel et al. 2017). Besides end-to-end neurorrhaphy, fibrin sealant can also be used for end-to-side repair as was demonstrated by Silva et al. (Silva et al. 2010), although results were not superior to conventional suturing (Silva et al. 2012b). Other possible experimental applications include reimplantation of lumbar ventral nerve roots after avulsion injury (Vidigal de Castro et al. 2016) and mitigation of pain after cervical nerve root injury (Weisshaar et al. 2011).

One advantageous feature of nerve repair with fibrin sealant is its easy application resulting in a shorter duration of the surgery (technical gain) (Smahel and Meyer 1995). Ornelas and colleagues reported a reduction of the necessary repair time to

about one third when using fibrin sealant (Ornelas et al. 2006a; Romano et al. 1991; Bozorg Grayeli et al. 2005; Bhandari 2013; Knox et al. 2013). This is especially relevant in case of an inexperienced surgeon scaling the steep learning curve of microsurgery (Whitlock et al. 2010). It is however a legitimate subject of debate if this shortcut is solely beneficial for an aspiring neuro- or plastic surgeon. An additional advantageous feature of fibrin sealant is avoidance of additional trauma to the nerve since no sutures have to be placed within its sheaths, for example, epi- and perineurium (Buchaim et al. 2015).

Thirdly, several studies reported that intraneural fibrosis, inflammatory processes, and formation of scar tissue were reduced to a minimum (biological gain) (Jin et al. 1990; Sameem et al. 2011; Leite et al. 2019). This may also be a fitting explanation for the reports of increased total number of nerve fibers after nerve repair with fibrin sealant (Felix et al. 2013) and greater amount of sprouting axons with an also significantly higher number of branches per regenerating axon in comparison to epineurial suture (Koulaxouzidis et al. 2015). In addition, application of fibrin sealant as a wrapping around the nerve prior to transection was reported as efficient to prevent protrusion of the axoplasm before performance of neurorrhaphy (Jou et al. 1999; Menovsky and Bartels 1999). A cuff of fibrin sealant can also be used to mold multiple cable grafts into a circular shape to facilitate neurorrhaphy by conventional suture (Draeger et al. 2017).

In contrast to these advantageous features, some authors reported reduced tensile strength and overall inferior results of fibrin sealant for repair of peripheral nerve injuries (Moy et al. 1988; Maragh et al. 1990; Cruz et al. 1986; Temple et al. 2004; Lin et al. 2010; Wang et al. 2018), while others reported equal or at least sufficient tensile strength compared to neurorrhaphy (Povlsen 1994; Erfanian et al. 2014). These contradicting results could be related to differences in terms of fibrin adhesive composition and preparation on the one hand and, on the other hand, in the choice of the respective animal and nerve injury model. Since Nishimura and colleagues found reduced tensile strength of fibrin sealant-based nerve repair when compared to neurorrhaphy until 2 weeks after surgery with equal strength afterwards, the observation period should be considered as an additional important factor regarding these observations (Nishimura et al. 2008). This may also be one of the underlying reasons that other experimental studies comparing fibrin sealant to neurorrhaphy did not report significant differences between both techniques (Farrag et al. 2007).

Isaacs and colleagues, who conducted the first cadaveric study to investigate tensile strength after nerve repair with fibrin sealant in human specimens, reported no differences between fibrin sealant and neurorrhaphy (Isaacs et al. 2008). Lastly, and in contrast with the original intention to limit fibrosis by replacing sutures, some authors reported fibrosis-promoting effects of fibrin sealant, mainly attributable to thrombin, factor XIII, and fibronectin (Herter et al. 1989).

In conclusion, there is still no consent regarding the efficacy of fibrin sealant in comparison to neurorrhaphy for coaptation of severed nerve ends. Although this question was subject to a multitude of animal studies, reports of successful nerve

coaptation with fibrin sealant in humans remain anecdotal in cases such as facial nerve repair (Choi et al. 2014; Ramos et al. 2015), nerve coaptation in phalloplasty (Salmeron-Gonzalez et al. 2019), and graft repair of the obturator nerve (Scaletta et al. 2019). Regarding this lack of randomized controlled trials in human patients, the use of fibrin sealant instead of conventional neurorrhaphy techniques remains a controversial subject of scientific debate (Sameem et al. 2011).

5.2 Fibrin Sealant for Enhancement of Neurorrhaphy Sites

Supplementation of neurorrhaphy with fibrin sealant to reduce the number of sutures aims to minimize foreign body reaction and scarring while providing sufficient tensile strength at the site of nerve repair. Moy et al. used fibrin sealant to enhance nerve repair by means of 2 epineurial sutures but were discouraged by their results. They concluded that this technique would not be a feasible alternative to suture repair at all (Moy et al. 1988). Bento and colleagues applied the same principle to repair the facial nerve in human patients (Bento and Miniti 1993). In contrast, they found the technique of enhancement of neurorrhaphy by fibrin sealant to be a safe and effective technique. This was further supported by studies and case reports, which investigated adjuvant application of fibrin sealant after neurorrhaphy and did not find any deleterious effects (Rafjah et al. 2013; Theberge and Ziccardi 2016) or even reported superior results when compared to suture alone (Leite et al. 2019). As mentioned before, these varying results could be related to different compositions of the used fibrin sealant and the experimental model used for the investigations. For example, differences in tensile stress due to joint movements between the tibial nerve and the facial nerve could be a plausible reason for differing histological and clinical results. In another study in rats, reduction of sutures by adding fibrin sealant to the neurorrhaphy site proved sufficient tensile strength to keep the nerve stumps attached to each other but did not result in better functional outcome when compared to “suture only” groups. It is noteworthy that the combination of one suture with fibrin sealant did not result in significantly improved tensile strength compared to one suture alone in this study (Erfanian et al. 2014). Therefore, there was no scientific evidence that adding fibrin sealant to a neurorrhaphy site has any benefit in regard to tensile strength. This changed in 2018 when Childe and colleagues used fibrin sealant to enhance conduit-assisted primary nerve repair in 90 cadaveric human nerve specimens. Since these authors reported significantly increased tensile strength in all groups with additional fibrin sealant besides sutures, they concluded that fibrin sealant can be used to reduce the number of necessary sutures and therefore limit the subsequent inflammatory response (Childe et al. 2018). Interestingly, studies which investigated use of fibrin sealant to coat porous nerve guidance conduits with the intention to prevent invasion of scar tissue reported deleterious results. In fact, the authors’ findings suggest increased attraction of nonneural cells by the coating which increased scar formation instead of preventing it (Bhatnagar et al. 2017).

5.3 Fibrin as a Carrier Matrix: In Vivo

Besides mechanical enhancement (technical gain) of neurorrhaphies, fibrin can also be used to enhance the site of nerve repair with cells, growth factors, and various other molecules (biological gain) to support nerve regeneration (Fig. 4). In contrast to other delivery systems, which allow a spatiotemporal gradient of drug delivery, e.g. miniosmotic pumps, use of fibrin as a carrier matrix neither requires implantation of foreign material within the body, nor does it depend on any sort of mechanical or chemical driving force (Tajdaran et al. 2016). As fibrin is degraded gradually by proteases, modification of its biological properties prior to implantation is a feasible tool to adjust the degradation time to individual needs and specifications (Lee et al. 2003, 2014). It also circumvents the problem of targeted delivery of the respective drug or cell, since systemic injection of those often results in loss of most of the cargo and repeated local injection can cause tissue damage or, in terms of nervous tissue, neuroma formation (Mozafari et al. 2018).

Yin et al. were the first to use fibrin to deliver proregenerative molecules to the site of nerve injury. They used fibrin sealant mixed with NT-4 in a rat model of sciatic nerve transection and immediate repair. Despite the fact that no data regarding the release duration of NT-4 was assessed, the authors found that NT-4 significantly increased nerve regeneration in their model (Yin et al. 2001). Using drug-loaded microspheres embedded within a fibrin gel, Tajdaran reported twofold increased nerve regeneration in comparison to unenhanced nerve suture in a rat model of delayed sciatic nerve repair (Tajdaran et al. 2016). Supplementation of a decellularized nerve graft with MSCs embedded in fibrin sealant resulted in significantly improved nerve regeneration in comparison to nonsupplemented nerve grafting in rats. Therefore, this approach was deemed a suitable alternative to autograft repair by Zhao and colleagues. Interestingly, the authors found that MSCs encapsulated in fibrin sealant showed reduced viability to nonencapsulated MSCs *in vitro* (Zhao et al. 2014). Adipose tissue-derived stem cells (ADSCs) supplied within a fibrin matrix also resulted in favorable results compared to neurorrhaphy alone in rat models of sciatic nerve transection (Reichenberger et al. 2016) and segmental injury with autograft repair (Masgutov et al. 2019). A further advance of this cell-based approach was undertaken by adding hESCs, which were genetically altered to overexpress FGF2, embedded within fibrin, to a 5-mm autograft repair of the sciatic nerve in mice. Although viability of the cells was not assessed by the authors, they reported improved sensory recovery in the group with transgenic hESCs embedded within fibrin sealant (Mozafari et al. 2018).

5.4 Fibrin as a Filler for Nerve Guidance Conduits

Use of fibrin as filler matrix within a conduit was pioneered by Galla and colleagues in 2004, which used a fibrin matrix enriched with Schwann cells and neurotrophic factors to bridge a 10-mm gap in the facial nerve of rats. However, they reported inferior results of their approach compared to autograft repair (Galla et al. 2004). Choi et al.

used an autologous vein filled with a fibrin matrix to bridge 17-mm peroneal nerve defects in rabbits. Although the authors did not include a control group treated with an autologous nerve graft, they deemed fibrin the optimal substance with which to fill a hollow conduit (Choi et al. 2005; Marcol et al. 2005). This was supported by findings of other authors who also used conduits filled with fibrin to bridge segmental nerve defects in rodent models with results at least equal or even superior to autologous nerve grafts (Khojasteh et al. 2016; Roth et al. 2017; Nakayama et al. 2007a, b; Lichtenfels et al. 2013). Schuh et al. used rolled collagen-fibrin engineered neural tissue inside a silicone tube to bridge an 8-mm defect of the sciatic nerve in rats. They found increased numbers of axons in the mid-graft as well as in the distal nerve compared to conduits filled with collagen only (Schuh et al. 2018). Carriel and colleagues added mesenchymal stem cells to a fibrin-agarose gel within a collagen nerve conduit, which resulted in enhanced nerve regeneration in comparison to conduits filled with fibrin-agarose gels alone (Carriel et al. 2013), but was inferior in comparison to autograft repair (Chato-Astrain et al. 2018). Besides cells, fibrin can also be enhanced using neurotrophic factors such as NGF (Lee et al. 2003; Gao et al. 2008; Ma et al. 2013) or GDNF (Wood et al. 2009, 2013; Jubran and Widenfalk 2003). Delivery of other molecules such as the oxygen carrier perfluorotributylamine (PFTBA) is also possible via fibrin, resulting in improved nerve regeneration after sciatic nerve neurotmesis in rats (Wang et al. 2013). Besides modification by means of embedded cells or molecules, another approach was investigated by Du et al. who aligned a fibrin hydrogel hierarchically via electrospinning and molecular self-assembly to mimic the natural fibrin cable (Yao et al. 2016; Du et al. 2017). Filled within a chitosan conduit, the hydrogel was then used to bridge a 10-mm gap of the sciatic nerve in rats, proving almost equal to an autologous nerve graft.

5.5 Conduits and Scaffolds

The first use of a fibrin conduit wrapped around a nerve was reported by Michael et al. in 1943, who used it in a patient who suffered a military injury of the sciatic nerve (Michael and Abbott 1943). While the authors reported improved clinical outcome after fibrin wrapping of the nerve, they also stressed the lack of irritation or foreign body reaction, emphasizing the absence of adverse effects of their new treatment approach. Singer et al. published their findings after use of a fibrin film for nerve repair of sciatic nerves in rabbits. Even though they reported successful healing of the nerves, they stressed the reduced tensile strength at the repair site (Singer 1945). Griffiths and colleagues used a nerve tube made of fibrin and collagen which proved equal to direct coaptation in a 5-mm defect model but was inferior in a 10-mm defect. These authors also recommended to avoid tension on the suture due to reduced tensile strength (Griffiths et al. 1990). Other authors made use of fibrin conduits for experimental nerve repair and found the results to be satisfying enough to recommend such conduits as a suitable alternative to successfully bridge non-critical segmental peripheral nerve injuries (Pettersson et al. 2010; Wang et al. 2018; Nicoli Aldini et al. 1995; Kalbermatten et al. 2009). However, Longo and colleagues

reported inferior functional recovery when comparing autograft repair and fibrin conduits in the sciatic nerve model of the rat (Longo et al. 2016). On the other hand, it is noteworthy that fibrin conduits were reported as inferior to autologous nerve grafts for reconstruction of critical size defects when they are not enhanced with additional proregenerative cells (Pettersson et al. 2011). In this respect, conduits can be enriched with various cells, such as Schwann cells or stem cells to further support nerve regeneration (di Summa et al. 2010, 2011; McGrath et al. 2012; Cartarozzi et al. 2015). Saller et al. used a fibrin conduit carrying ADSCs to enhance nerve regeneration after end-to-end neurorrhaphy in a rat model and reported significantly better functional recovery compared to the group which received no additional treatment besides suture of the nerve ends (Saller et al. 2018).

6 Conclusions

Applications of fibrin and fibrin sealants have been steadily increasing both qualitatively and quantitatively throughout the last 80 years. Originally intended to replace sutures for nerve repair to limit fibrosis, fibrin was also tested in combination with conventional neurorrhaphy to substitute a minimal number of sutures. Given its immunological inertness and ease of biological modifications, fibrin can be used as a scaffold for in vivo release of neurotrophins, cells, and other proregenerative molecules. However, in contrast to the myriad of published experimental studies in animals with differing results, human studies are sparse. This might be the underlying reason that fibrin, despite its versatile and marvelous possible applications, has not yet tackled the final frontier of routine use in human patients with peripheral nerve injuries. The authors of this chapter hope that further refinements in both biological and technical aspects will further advance utilization and reliability of fibrin in peripheral nerve regeneration and repair, eventually fulfilling Sir Herbert Seddon's prediction from eight decades ago.

References

- Adel M et al (2017) Suture versus fibrin glue microneural anastomosis of the femoral nerve in Sprague Dawley rat model. A comparative experimental assessment of the clinical, histological and statistical features. *Acta Chir Plast* 59(2):65–71
- Aebischer P, Guenard V, Valentini RF (1990) The morphology of regenerating peripheral nerves is modulated by the surface microgeometry of polymeric guidance channels. *Brain Res* 531(1–2):211–218
- Ahmad E et al (2015) Fibrin matrices: the versatile therapeutic delivery systems. *Int J Biol Macromol* 81:121–136
- Ahmed TA, Dare EV, Hincke M (2008) Fibrin: a versatile scaffold for tissue engineering applications. *Tissue Eng Part B Rev* 14(2):199–215
- AKassoglou K, Strickland S (2002) Nervous system pathology: the fibrin perspective. *Biol Chem* 383(1):37–45
- AKassoglou K et al (2002) Fibrin inhibits peripheral nerve remyelination by regulating Schwann cell differentiation. *Neuron* 33(6):861–875

- Akassoglou K et al (2003) Fibrin is a regulator of Schwann cell migration after sciatic nerve injury in mice. *Neurosci Lett* 338(3):185–188
- Aleman MM et al (2014) Fibrinogen and red blood cells in venous thrombosis. *Thromb Res* 133 (Suppl 1):S38–S40
- Alshehri OM et al (2015) Fibrin activates GPVI in human and mouse platelets. *Blood* 126 (13):1601–1608
- Altieri DC et al (1990) A unique recognition site mediates the interaction of fibrinogen with the leukocyte integrin Mac-1 (CD11b/CD18). *J Biol Chem* 265(21):12119–12122
- Ariens RA et al (2002) Role of factor XIII in fibrin clot formation and effects of genetic polymorphisms. *Blood* 100(3):743–754
- Astrup T (1958) The haemostatic balance. *Thromb Diath Haemorrh* 2(3–4):347–357
- Attar BM et al (2012) Effectiveness of fibrin adhesive in facial nerve anastomosis in dogs compared with standard microsutures technique. *J Oral Maxillofac Surg* 70(10):2427–2432
- Bagoly Z et al (2012) Factor XIII, clot structure, thrombosis. *Thromb Res* 129(3):382–387
- Barbizan R et al (2013) Motor recovery and synaptic preservation after ventral root avulsion and repair with a fibrin sealant derived from snake venom. *PLoS One* 8(5):e63260
- Barnes JM, Przybyla L, Weaver VM (2017) Tissue mechanics regulate brain development, homeostasis and disease. *J Cell Sci* 130(1):71–82
- Bayram B et al (2018) Effects of platelet-rich fibrin membrane on sciatic nerve regeneration. *J Craniofac Surg* 29(3):e239–e243
- Becker CM, Gueuning CO, Graff GL (1984) Sutures of fibrin glue for divided rat nerves. Schwann cell and muscle metabolism. *J Reconstr Microsurg* 1(2):139–145
- Belanger K et al (2016) Recent strategies in tissue engineering for guided peripheral nerve regeneration. *Macromol Biosci* 16(4):472–481
- Belkas JS, Shoichet MS, Midha R (2004) Peripheral nerve regeneration through guidance tubes. *Neurol Res* 26(2):151–160
- Benkherourou M et al (2000) Quantification and macroscopic modeling of the nonlinear viscoelastic behavior of strained gels with varying fibrin concentrations. *IEEE Trans Biomed Eng* 47 (11):1465–1475
- Bennett JS (2005) Structure and function of the platelet integrin α IIb β 3. *J Clin Invest* 115 (12):3363–3369
- Bensaid W et al (2003) A biodegradable fibrin scaffold for mesenchymal stem cell transplantation. *Biomaterials* 24(14):2497–2502
- Bento RF, Miniti A (1993) Anastomosis of the intratemporal facial nerve using fibrin tissue adhesive. *Ear Nose Throat J* 72(10):663
- Bento AR et al (2017) Three-dimensional culture of single embryonic stem-derived neural/stem progenitor cells in fibrin hydrogels: neuronal network formation and matrix remodelling. *J Tissue Eng Regen Med* 11(12):3494–3507
- Bergel S (1909) Ueber Wirkungen des Fibrins. *Dtsch Med Wochenschr* 35(15):663–665
- Bergmeister KD et al (2020) Acute and long-term costs of 268 peripheral nerve injuries in the upper extremity. *PLoS One* 15(4):e0229530
- Bevilacqua MP (1993) Endothelial-leukocyte adhesion molecules. *Annu Rev Immunol* 11:767–804
- Bhandari PS (2013) Use of fibrin glue in the repair of brachial plexus and peripheral nerve injuries. *Indian J Neurotrauma* 10(1):30–32
- Bhang SH et al (2007) Controlled release of nerve growth factor from fibrin gel. *J Biomed Mater Res A* 80(4):998–1002
- Bhatnagar D et al (2017) Fibrin glue as a stabilization strategy in peripheral nerve repair when using porous nerve guidance conduits. *J Mater Sci Mater Med* 28(5):79
- Biscola NP et al (2017) Multiple uses of fibrin sealant for nervous system treatment following injury and disease. *J Venom Anim Toxins Incl Trop Dis* 23:13
- Blomback B, Hessel B, Hogg D (1976) Disulfide bridges in nh2-terminal part of human fibrinogen. *Thromb Res* 8(5):639–658
- Blomback B et al (1978) A two-step fibrinogen – fibrin transition in blood coagulation. *Nature* 275 (5680):501–505

- Blomback B et al (1994) Fibrin in human plasma: gel architectures governed by rate and nature of fibrinogen activation. *Thromb Res* 75(5):521–538
- Bozorg Grayeli A et al (2005) Long-term functional outcome in facial nerve graft by fibrin glue in the temporal bone and cerebellopontine angle. *Eur Arch Otorhinolaryngol* 262(5):404–407
- Breen A, O'Brien T, Pandit A (2009) Fibrin as a delivery system for therapeutic drugs and biomolecules. *Tissue Eng Part B Rev* 15(2):201–214
- Brown AC, Barker TH (2014) Fibrin-based biomaterials: modulation of macroscopic properties through rational design at the molecular level. *Acta Biomater* 10(4):1502–1514
- Buchaim RL et al (2015) Effect of low-level laser therapy (LLLT) on peripheral nerve regeneration using fibrin glue derived from snake venom. *Injury* 46(4):655–660
- Burgess WH et al (1990) Possible dissociation of the heparin-binding and mitogenic activities of heparin-binding (acidic fibroblast) growth factor-1 from its receptor-binding activities by site-directed mutagenesis of a single lysine residue. *J Cell Biol* 111(5 Pt 1):2129–2138
- Carr ME Jr, Gabriel DA, McDonagh J (1986) Influence of Ca^{2+} on the structure of reptilase-derived and thrombin-derived fibrin gels. *Biochem J* 239(3):513–516
- Carriel V et al (2013) Combination of fibrin-agarose hydrogels and adipose-derived mesenchymal stem cells for peripheral nerve regeneration. *J Neural Eng* 10(2):026022
- Cartarozzi LP et al (2015) Mesenchymal stem cells engrafted in a fibrin scaffold stimulate Schwann cell reactivity and axonal regeneration following sciatic nerve tubulization. *Brain Res Bull* 112:14–24
- Cesarman-Maus G, Hajjar KA (2005) Molecular mechanisms of fibrinolysis. *Br J Haematol* 129(3):307–321
- Chandrababu K, Senan M, Krishnan LK (2020) Exploitation of fibrin-based signaling niche for deriving progenitors from human adipose-derived mesenchymal stem cells towards potential neural engineering applications. *Sci Rep* 10(1):7116
- Chapin JC, Hajjar KA (2015) Fibrinolysis and the control of blood coagulation. *Blood Rev* 29(1):17–24
- Charo IF, Bekeart LS, Phillips DR (1987) Platelet glycoprotein IIb-IIIa-like proteins mediate endothelial cell attachment to adhesive proteins and the extracellular matrix. *J Biol Chem* 262(21):9935–9938
- Chato-Astrain J et al (2018) In vivo evaluation of nanostructured fibrin-agarose hydrogels with mesenchymal stem cells for peripheral nerve repair. *Front Cell Neurosci* 12:501
- Cheresh DA et al (1989) Recognition of distinct adhesive sites on fibrinogen by related integrins on platelets and endothelial cells. *Cell* 58(5):945–953
- Chernysh IN, Nagaswami C, Weisel JW (2011) Visualization and identification of the structures formed during early stages of fibrin polymerization. *Blood* 117(17):4609–4614
- Childe JR et al (2018) Fibrin glue increases the tensile strength of conduit-assisted primary digital nerve repair. *Hand (NY)* 13(1):45–49
- Choi BH et al (2005) Autologous fibrin glue in peripheral nerve regeneration in vivo. *Microsurgery* 25(6):495–499
- Choi KS et al (2014) Preservation of facial nerve function repaired by using fibrin glue-coated collagen fleece for a totally transected facial nerve during vestibular schwannoma surgery. *J Korean Neurosurg Soc* 55(4):208–211
- Christman KL et al (2004) Fibrin glue alone and skeletal myoblasts in a fibrin scaffold preserve cardiac function after myocardial infarction. *Tissue Eng* 10(3–4):403–409
- Chung DW, Davie EW (1984) Gamma and gamma' chains of human fibrinogen are produced by alternative mRNA processing. *Biochemistry* 23(18):4232–4236
- Clark RA (2001) Fibrin and wound healing. *Ann N Y Acad Sci* 936:355–367
- Clark RA et al (1996) Transient functional expression of $\alpha V\beta 3$ on vascular cells during wound repair. *Am J Pathol* 148(5):1407–1421
- Clemetson KJ (2012) Platelets and primary haemostasis. *Thromb Res* 129(3):220–224
- Cohen C, Revel JP, Kucera J (1963) Paracrystalline forms of fibrinogen. *Science* 141(3579):436–438
- Collet JP et al (2000) Influence of fibrin network conformation and fibrin fiber diameter on fibrinolysis speed: dynamic and structural approaches by confocal microscopy. *Arterioscler Thromb Vasc Biol* 20(5):1354–1361

- Collet JP et al (2006) Altered fibrin architecture is associated with hypofibrinolysis and premature coronary atherothrombosis. *Arterioscler Thromb Vasc Biol* 26(11):2567–2573
- Costa-Filho R et al (2016) Over 50 years of fibrinogen concentrate. *Clin Appl Thromb Hemost* 22(2):109–114
- Cronje HT et al (2017) Candidate gene analysis of the fibrinogen phenotype reveals the importance of polygenic co-regulation. *Matrix Biol* 60–61:16–26
- Cruz NI, Debs N, Fiol RE (1986) Evaluation of fibrin glue in rat sciatic nerve repairs. *Plast Reconstr Surg* 78(3):369–373
- Davie EW, Ratnoff OD (1964) Waterfall sequence for intrinsic blood clotting. *Science* 145(3638):1310–1312
- Davie EW, Fujikawa K, Kisiel W (1991) The coagulation cascade: initiation, maintenance, and regulation. *Biochemistry* 30(43):10363–10370
- Davis G, Curtin CM (2016) Management of Pain in complex nerve injuries. *Hand Clin* 32(2):257–262
- Davis KD, Taylor KS, Anastakis DJ (2011) Nerve injury triggers changes in the brain. *Neuroscientist* 17(4):407–422
- de la Puente P, Ludena D (2014) Cell culture in autologous fibrin scaffolds for applications in tissue engineering. *Exp Cell Res* 322(1):1–11
- di Summa PG et al (2010) Adipose-derived stem cells enhance peripheral nerve regeneration. *J Plast Reconstr Aesthet Surg* 63(9):1544–1552
- di Summa PG et al (2011) Long-term in vivo regeneration of peripheral nerves through bioengineered nerve grafts. *Neuroscience* 181:278–291
- Dohan DM et al (2006) Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101(3):e45–e50
- Doolittle RF, Chen R, Lau F (1971) Hybrid fibrin: proof of the intermolecular nature of – crosslinking units. *Biochem Biophys Res Commun* 44(1):94–100
- Doolittle RF, Spraggon G, Everse SJ (1997) Evolution of vertebrate fibrin formation and the process of its dissolution. *Ciba Found Symp* 212:4–17; discussion 17–23
- Draeger RW, Bynum DK Jr, Patterson JMM (2017) Simplified cable nerve grafting with nerve-cutting guides and fibrin glue. *J Hand Microsurg* 9(3):167–169
- Dresdale A et al (1985) Preparation of fibrin glue from single-donor fresh-frozen plasma. *Surgery* 97(6):750–755
- Du J et al (2017) Prompt peripheral nerve regeneration induced by a hierarchically aligned fibrin nanofiber hydrogel. *Acta Biomater* 55:296–309
- Dubey N, Letourneau PC, Tranquillo RT (2001) Neuronal contact guidance in magnetically aligned fibrin gels: effect of variation in gel mechano-structural properties. *Biomaterials* 22(10):1065–1075
- Duong H, Wu B, Tawil B (2009) Modulation of 3D fibrin matrix stiffness by intrinsic fibrinogen-thrombin compositions and by extrinsic cellular activity. *Tissue Eng Part A* 15(7):1865–1876
- Dvorak HF et al (1987) Fibrin containing gels induce angiogenesis. Implications for tumor stroma generation and wound healing. *Lab Invest* 57(6):673–686
- Edgar JM, Robinson M, Willerth SM (2017) Fibrin hydrogels induce mixed dorsal/ventral spinal neuron identities during differentiation of human induced pluripotent stem cells. *Acta Biomater* 51:237–245
- Egloff DV, Narakas A (1983) Nerve anastomoses with human fibrin. Preliminary clinical report (56 cases). *Ann Chir Main* 2(2):101–115
- Engler AJ et al (2006) Matrix elasticity directs stem cell lineage specification. *Cell* 126(4):677–689
- Erfanian R et al (2014) Comparison of a new single-donor human fibrin adhesive with suture for posterior tibial nerve repair in rat: biomechanical resistance and functional analysis. *Chin J Traumatol* 17(3):146–152
- Espitia Jaimes C, Fish RJ, Neerman-Arbez M (2018) Local chromatin interactions contribute to expression of the fibrinogen gene cluster. *J Thromb Haemost* 16(10):2070–2082
- Everse SJ et al (1998) Crystal structure of fragment double-D from human fibrin with two different bound ligands. *Biochemistry* 37(24):8637–8642

- Ewald SG, Beckmann-Fries V (2017) Rehabilitation following peripheral nerve injury. In: Haastert-Talini K, Assmus H, Antoniadis G (eds) Modern concepts of peripheral nerve repair. Springer International Publishing, Cham, pp 109–125
- Farrag TY et al (2007) Effect of platelet rich plasma and fibrin sealant on facial nerve regeneration in a rat model. *Laryngoscope* 117(1):157–165
- Fattahi T, Mohan M, Caldwell GT (2004) Clinical applications of fibrin sealants. *J Oral Maxillofac Surg* 62(2):218–224
- Felix SP et al (2013) Comparison between suture and fibrin glue on repair by direct coaptation or tubulization of injured mouse sciatic nerve. *Microsurgery* 33(6):468–477
- Fish RJ, Neerman-Arbez M (2012) Fibrinogen gene regulation. *Thromb Haemost* 108(3):419–426
- Fogelson AL, Keener JP (2010) Toward an understanding of fibrin branching structure. *Phys Rev E Stat Nonlinear Soft Matter Phys* 81(5 Pt 1):051922
- Forrester JM (1995) Malpighi's De polypo cordis: an annotated translation. *Med Hist* 39(4):477–492
- Forsgren M et al (1987) Molecular cloning and characterization of a full-length cDNA clone for human plasminogen. *FEBS Lett* 213(2):254–260
- Foster CH et al (2019) Trends and cost-analysis of lower extremity nerve injury using the National Inpatient Sample. *Neurosurgery* 85(2):250–256
- Fowler WE et al (1980) Electron microscopy of plasmonic fragments of human fibrinogen as related to trinodular structure of the intact molecule. *J Clin Invest* 66(1):50–56
- Gaffney PJ et al (1988) Monoclonal antibodies to crosslinked fibrin degradation products (XL-FDP). II. Evaluation in a variety of clinical conditions. *Br J Haematol* 68(1):91–96
- Gailani D, Renne T (2007) Intrinsic pathway of coagulation and arterial thrombosis. *Arterioscler Thromb Vasc Biol* 27(12):2507–2513
- Gailit J et al (1997) Human fibroblasts bind directly to fibrinogen at RGD sites through integrin $\alpha(v)\beta3$. *Exp Cell Res* 232(1):118–126
- Galla TJ et al (2004) Fibrin/Schwann cell matrix in poly-epsilon-caprolactone conduits enhances guided nerve regeneration. *Int J Artif Organs* 27(2):127–136
- Gao C et al (2008) Sciatic nerve regeneration in rats stimulated by fibrin glue containing nerve growth factor: an experimental study. *Injury* 39(12):1414–1420
- Gestring GF, Lerner R (1983) Autologous fibrinogen for tissue-adhesion, hemostasis and embolization. *Vasc Surg* 17(5):294–304
- Ghebrehewet B, Silverberg M, Kaplan AP (1981) Activation of the classical pathway of complement by Hageman factor fragment. *J Exp Med* 153(3):665–676
- Godal HC (1969) Delayed fibrin polymerization due to removal of calcium ions. *Scand J Clin Lab Invest* 24(1):29–33
- Graeff H, Hafter R (1982) Detection and relevance of crosslinked fibrin derivatives in blood. *Semin Thromb Hemost* 8(1):57–68
- Grainger DJ et al (1995) Release and activation of platelet latent TGF-beta in blood clots during dissolution with plasmin. *Nat Med* 1(9):932–937
- Greiling D, Clark RA (1997) Fibronectin provides a conduit for fibroblast transmigration from collagenous stroma into fibrin clot provisional matrix. *J Cell Sci* 110(Pt 7):861–870
- Griffiths R, Horch K, Stensaas L (1990) A collagen and fibrin tube for nerve repair. *Restor Neurol Neurosci* 1(5):339–346
- Gurewicz V et al (1984) Effective and fibrin-specific clot lysis by a zymogen precursor form of urokinase (pro-urokinase). A study in vitro and in two animal species. *J Clin Invest* 73(6):1731–1739
- Haase J et al (1986) Use of fibrin seal (Tisseel/Tissucol) in peripheral nerve surgery: an experimental rat model. Springer, Berlin/Heidelberg
- Hantgan R et al (1980) Fibrin assembly: a comparison of electron microscopic and light scattering results. *Thromb Haemost* 44(3):119–124
- Hardy JJ, Carrell NA, McDonagh J (1983) Calcium ion functions in fibrinogen conversion to fibrin. *Ann N Y Acad Sci* 408:279–287
- Hartley H (1951) Origin of the word 'protein'. *Nature* 168(4267):244–244

- Heemskerk JW, Bevers EM, Lindhout T (2002) Platelet activation and blood coagulation. *Thromb Haemost* 88(2):186–193
- Heher P et al (2015) A novel bioreactor for the generation of highly aligned 3D skeletal muscle-like constructs through orientation of fibrin via application of static strain. *Acta Biomater* 24:251–265
- Heher P et al (2018a) An overview of surgical sealant devices: current approaches and future trends. *Expert Rev Med Devices* 15(10):747–755
- Heher P et al (2018b) Fibrin-based delivery strategies for acute and chronic wound healing. *Adv Drug Deliv Rev* 129:134–147
- Hermans J, McDonagh J (1982) Fibrin: structure and interactions. *Semin Thromb Hemost* 8(1):11–24
- Herter T, Anagnostopoulos-Schleep J, Bennefeld H (1989) [The effect of fibrin gluing and its important components on fibrosis of nerve anastomoses]. *Unfallchirurgie*. 15(5):221–229
- Hettasch JM, Bolyard MG, Lord ST (1992) The residues AGDV of recombinant gamma chains of human fibrinogen must be carboxy-terminal to support human platelet aggregation. *Thromb Haemost* 68(6):701–706
- Hoepflich PD Jr, Doolittle RF (1983) Dimeric half-molecules of human fibrinogen are joined through disulfide bonds in an antiparallel orientation. *Biochemistry* 22(9):2049–2055
- Hoffman-Kim D, Mitchel JA, Bellamkonda RV (2010) Topography, cell response, and nerve regeneration. *Annu Rev Biomed Eng* 12:203–231
- Hoover-Plow J (2010) Does plasmin have anticoagulant activity? *Vasc Health Risk Manag* 6:199–205
- Hoylaerts M et al (1982) Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin. *J Biol Chem* 257(6):2912–2919
- Huckhagel T et al (2018a) Nerve injury in severe trauma with upper extremity involvement: evaluation of 49,382 patients from the TraumaRegister DGU(R) between 2002 and 2015. *Scand J Trauma Resusc Emerg Med* 26(1):76
- Huckhagel T et al (2018b) Nerve trauma of the lower extremity: evaluation of 60,422 leg injured patients from the TraumaRegister DGU[®] between 2002 and 2015. *Scand J Trauma Resusc Emerg Med* 26(1):40–40
- Isaacs JE et al (2008) Comparative analysis of biomechanical performance of available “nerve glues”. *J Hand Surg Am* 33(6):893–899
- Iuan FC et al (1995) Reparation of peripheral nerves with fibrin glue prepared from snake venom. Preliminary results. *Sao Paulo Med J* 113(5):1000–1002
- Jakob W, Zipper J, Jentzsch ED (1982) Is the formation of fibrin a necessary event for the initiation of angiogenic responses in the chick chorioallantoic membrane? *Exp Pathol* 21(4):251–262
- Janmey PA, Winer JP, Weisel JW (2009) Fibrin gels and their clinical and bioengineering applications. *J R Soc Interface* 6(30):1–10
- Jansen JW et al (1987) Influence of factor XIIIa activity on human whole blood clot lysis in vitro. *Thromb Haemost* 57(2):171–175
- Janus TJ et al (1983) Promotion of thrombin-catalyzed activation of factor XIII by fibrinogen. *Biochemistry* 22(26):6269–6272
- Jennewein C et al (2011) Novel aspects of fibrin(ogen) fragments during inflammation. *Mol Med* 17(5–6):568–573
- Jeon O et al (2005) Control of basic fibroblast growth factor release from fibrin gel with heparin and concentrations of fibrinogen and thrombin. *J Control Release* 105(3):249–259
- Jeon O et al (2008) Long-term delivery enhances in vivo osteogenic efficacy of bone morphogenetic protein-2 compared to short-term delivery. *Biochem Biophys Res Commun* 369(2):774–780
- Jin Y et al (1990) Comparative experimental study of nerve repairs by classical suture or biological adhesive. *Neurochirurgie* 36(6):378–382
- Johnson PJ et al (2010) Controlled release of neurotrophin-3 and platelet-derived growth factor from fibrin scaffolds containing neural progenitor cells enhances survival and differentiation into neurons in a subacute model of SCI. *Cell Transplant* 19(1):89–101
- Jou IM et al (1999) The influence of delay and the effect of fibrin sealant on the cut surface of the peripheral nerve. An experimental study in the rat. *J Hand Surg Br* 24(6):707–711

- Jubran M, Widenfalk J (2003) Repair of peripheral nerve transections with fibrin sealant containing neurotrophic factors. *Exp Neurol* 181(2):204–212
- Juliano RL, Haskill S (1993) Signal transduction from the extracellular matrix. *J Cell Biol* 120(3):577–585
- Kalafatis M et al (1997) The regulation of clotting factors. *Crit Rev Eukaryot Gene Expr* 7(3):241–280
- Kalbermatten DF et al (2008) Fibrin matrix for suspension of regenerative cells in an artificial nerve conduit. *J Plast Reconstr Aesthet Surg* 61(6):669–675
- Kalbermatten DF et al (2009) New fibrin conduit for peripheral nerve repair. *J Reconstr Microsurg* 25(1):27–33
- Kaplan HM, Mishra P, Kohn J (2015) The overwhelming use of rat models in nerve regeneration research may compromise designs of nerve guidance conduits for humans. *J Mater Sci Mater Med* 26(8):226
- Karsy M et al (2019) Trends and cost analysis of upper extremity nerve injury using the national (Nationwide) inpatient sample. *World Neurosurg* 123:e488–e500
- Kasai S et al (1985) Primary structure of single-chain pro-urokinase. *J Biol Chem* 260(22):12382–12389
- Khalafallah A et al (2014) Evaluation of the innovance d-dimer assay for the diagnosis of disseminated intravascular coagulopathy in different clinical settings. *Clin Appl Thromb Hemost* 20(1):91–97
- Khojasteh A et al (2016) The effect of a platelet-rich fibrin conduit on neurosensory recovery following inferior alveolar nerve lateralization: a preliminary clinical study. *Int J Oral Maxillofac Surg* 45(10):1303–1308
- Kirschbaum NE, Budzynski AZ (1990) A unique proteolytic fragment of human fibrinogen containing the a alpha COOH-terminal domain of the native molecule. *J Biol Chem* 265(23):13669–13676
- Knighton DR et al (1982) Role of platelets and fibrin in the healing sequence: an in vivo study of angiogenesis and collagen synthesis. *Ann Surg* 196(4):379–388
- Knox CJ et al (2013) Facial nerve repair: fibrin adhesive coaptation versus epineurial suture repair in a rodent model. *Laryngoscope* 123(7):1618–1621
- Koehn JA et al (1983) Sequence of plasmin proteolysis at the NH₂-terminus of the b beta-chain of human fibrinogen. *Anal Biochem* 133(2):502–510
- Koulaxouzidis G, Reim G, Witzel C (2015) Fibrin glue repair leads to enhanced axonal elongation during early peripheral nerve regeneration in an in vivo mouse model. *Neural Regen Res* 10(7):1166–1171
- Kuderna H (1976) Clinical application of nerve-anastomoses adhesion using fibrinogen. *Fortschr Kiefer Gesichtschir* 21:135
- Landers ZA et al (2018) The psychological impact of adult traumatic brachial plexus injury. *J Hand Surg Am* 43(10):950.e1–950.e6
- Lasne D, Jude B, Susen S (2006) From normal to pathological hemostasis. *Can J Anaesth* 53(6 Suppl):S2–S11
- Laudano AP, Doolittle RF (1978) Synthetic peptide derivatives that bind to fibrinogen and prevent the polymerization of fibrin monomers. *Proc Natl Acad Sci USA* 75(7):3085–3089
- Laurens N, Koolwijk P, de Maat MP (2006) Fibrin structure and wound healing. *J Thromb Haemost* 4(5):932–939
- Law RH et al (2012) The X-ray crystal structure of full-length human plasminogen. *Cell Rep* 1(3):185–190
- Lawrence DA (1997) The serpin-proteinase complex revealed. *Nat Struct Biol* 4(5):339–341
- Le Beau JM, Ellisman MH, Powell HC (1988) Ultrastructural and morphometric analysis of long-term peripheral nerve regeneration through silicone tubes. *J Neurocytol* 17(2):161–172
- Lee AC et al (2003) Controlled release of nerve growth factor enhances sciatic nerve regeneration. *Exp Neurol* 184(1):295–303
- Lee JY et al (2014) Controlled release of nerve growth factor from heparin-conjugated fibrin gel within the nerve growth factor-delivering implant. *J Korean Assoc Oral Maxillofac Surg* 40(1):3–10

- Leidy EM et al (1990) Enhanced thrombolysis by a factor XIIIa inhibitor in a rabbit model of femoral artery thrombosis. *Thromb Res* 59(1):15–26
- Leite APS et al (2019) Heterologous fibrin sealant potentiates axonal regeneration after peripheral nerve injury with reduction in the number of suture points. *Injury* 50(4):834–847
- Levy JH, Greenberg C (2013) Biology of factor XIII and clinical manifestations of factor XIII deficiency. *Transfusion* 53(5):1120–1131
- Lewis SD et al (1985) Regulation of formation of factor XIIIa by its fibrin substrates. *Biochemistry* 24(24):6772–6777
- Lichtenfels M et al (2013) Effect of platelet rich plasma and platelet rich fibrin on sciatic nerve regeneration in a rat model. *Microsurgery* 33(5):383–390
- Lijnen HR et al (1986) Activation of plasminogen by pro-urokinase. I. Mechanism. *J Biol Chem* 261(3):1253–1258
- Lin KL et al (2010) DuraSeal as a ligature in the anastomosis of rat sciatic nerve gap injury. *J Surg Res* 161(1):101–110
- Litvinov RI et al (2005) Polymerization of fibrin: specificity, strength, and stability of knob-hole interactions studied at the single-molecule level. *Blood* 106(9):2944–2951
- Litvinov RI et al (2007) Direct evidence for specific interactions of the fibrinogen alphaC-domains with the central E region and with each other. *Biochemistry* 46(31):9133–9142
- Liu HM (1992) The role of extracellular matrix in peripheral nerve regeneration: a wound chamber study. *Acta Neuropathol* 83(5):469–474
- Longo MV et al (2016) Comparisons of the results of peripheral nerve defect repair with fibrin conduit and autologous nerve graft: an experimental study in rats. *Microsurgery* 36(1):59–65
- Longstaff C, Kolev K (2015) Basic mechanisms and regulation of fibrinolysis. *J Thromb Haemost* 13(Suppl 1):S98–S105
- Lorand L (2001) Factor XIII: structure, activation, and interactions with fibrinogen and fibrin. *Ann N Y Acad Sci* 936:291–311
- Lorand L, Konishi K (1964) Activation of the fibrin stabilizing factor of plasma by thrombin. *Arch Biochem Biophys* 105:58–67
- Lundborg G (1982a) [Regeneration after peripheral nerve injury – a biological and clinical problem]. *Lakartidningen*. 79(20):2013–2017
- Lundborg G (1982b) Regeneration of peripheral nerves – a biological and surgical problem. *Scand J Plast Reconstr Surg Suppl* 19:38–44
- Lundborg G et al (1982a) Nerve regeneration across an extended gap: a neurobiological view of nerve repair and the possible involvement of neuronotrophic factors. *J Hand Surg Am* 7(6):580–587
- Lundborg G et al (1982b) In vivo regeneration of cut nerves encased in silicone tubes: growth across a six-millimeter gap. *J Neuropathol Exp Neurol* 41(4):412–422
- Lundborg G et al (1982c) Nerve regeneration in silicone chambers: influence of gap length and of distal stump components. *Exp Neurol* 76(2):361–375
- Lundborg G, Longo FM, Varon S (1982d) Nerve regeneration model and trophic factors in vivo. *Brain Res* 232(1):157–161
- Ma S et al (2013) Sciatic nerve regeneration using a nerve growth factor-containing fibrin glue membrane. *Neural Regen Res* 8(36):3416–3422
- Macfarlane RG (1964) An enzyme Cascade in the blood clotting mechanism, and its function as a biochemical amplifier. *Nature* 202:498–499
- Mackman N, Tilley RE, Key NS (2007) Role of the extrinsic pathway of blood coagulation in hemostasis and thrombosis. *Arterioscler Thromb Vasc Biol* 27(8):1687–1693
- Macrae FL et al (2018) A fibrin biofilm covers blood clots and protects from microbial invasion. *J Clin Invest* 128(8):3356–3368
- Mafi P, Beikpour H (2006) Management and results of extensive volar wrist lacerations: “The Spaagheti wrist” in 124 patients during a 5 years period in 15th khordad hospital tehran. *Med J Islam Repub Iran* 20(1):5–7
- Makogonenko E et al (2002) Interaction of fibrin(ogen) with fibronectin: further characterization and localization of the fibronectin-binding site. *Biochemistry* 41(25):7907–7913

- Maragh H et al (1990) Morphofunctional evaluation of fibrin glue versus microsuture nerve repairs. *J Reconstr Microsurg* 6(4):331–337
- Marcol W et al (2005) Extracts obtained from predegenerated nerves improve functional recovery after sciatic nerve transection. *Microsurgery* 25(6):486–494
- Marder VJ, Shulman NR, Carroll WR (1969) High molecular weight derivatives of human fibrinogen produced by plasmin. I. Physicochemical and immunological characterization. *J Biol Chem* 244(8):2111–2119
- Marquardt LM, Sakiyama-Elbert SE (2013) Engineering peripheral nerve repair. *Curr Opin Biotechnol* 24(5):887–892
- Martins RS et al (2005a) Overall assessment of regeneration in peripheral nerve lesion repair using fibrin glue, suture, or a combination of the 2 techniques in a rat model. Which is the ideal choice? *Surg Neurol* 64(Suppl 1):S1:10–S1:16; discussion S1:16
- Martins RS et al (2005b) Electrophysiologic assessment of regeneration in rat sciatic nerve repair using suture, fibrin glue or a combination of both techniques. *Arq Neuropsiquiatr* 63(3A):601–604
- Masgutov R et al (2019) Adipose-derived mesenchymal stem cells applied in fibrin glue stimulate peripheral nerve regeneration. *Front Med (Lausanne)* 6:68
- Matras H et al (1972) Suture-free interfascicular nerve transplantation in animal experiments. *Wien Med Wochenschr* 122(37):517–523
- Matricardi P et al (2013) Interpenetrating polymer networks polysaccharide hydrogels for drug delivery and tissue engineering. *Adv Drug Deliv Rev* 65(9):1172–1187
- Matsuka YV et al (1996) Factor XIIIa-catalyzed cross-linking of recombinant alpha C fragments of human fibrinogen. *Biochemistry* 35(18):5810–5816
- Matsumoto K, Chikuba H (1966) Fibrin film as the covering material on the nerve anastomosis site. *Shujutsu* 20(12):1100–1104
- McGrath AM et al (2012) Fibrin conduit supplemented with human mesenchymal stem cells and immunosuppressive treatment enhances regeneration after peripheral nerve injury. *Neurosci Lett* 516(2):171–176
- McManus MC et al (2006) Mechanical properties of electrospun fibrinogen structures. *Acta Biomater* 2(1):19–28
- McManus MC et al (2007) Electrospun fibrinogen: feasibility as a tissue engineering scaffold in a rat cell culture model. *J Biomed Mater Res A* 81(2):299–309
- Meals CG, Chang J (2018) Ten tips to simplify the spaghetti wrist. *Plast Reconstr Surg Glob Open* 6(12):e1971
- Medved L, Nieuwenhuizen W (2003) Molecular mechanisms of initiation of fibrinolysis by fibrin. *Thromb Haemost* 89(3):409–419
- Medved L et al (1990) Electron microscope investigation of the early stages of fibrin assembly. Twisted protofibrils and fibers. *J Mol Biol* 216(3):503–509
- Menorca RM, Fussell TS, Elfar JC (2013) Nerve physiology: mechanisms of injury and recovery. *Hand Clin* 29(3):317–330
- Menovsky T, Bartels RH (1999) Stabilization and accurate trimming of nerve ends: practical use of fibrin glue: technical note. *Neurosurgery* 44(1):224–225; discussion 225–6
- Metelka M, Skala E, Fuchsova M (1962) Glueing of served peripheral nerves with plasma coagulum. *Rozhl Chir* 41:802–809
- Michael P, Abbott W (1943) The use of human fibrinogen in reconstructive surgery. *JAMA* 123(5):279–279
- Mihalyi E (1988) Clotting of bovine fibrinogen. Calcium binding to fibrin during clotting and its dependence on release of fibrinopeptide B. *Biochemistry* 27(3):967–976
- Moaddel M et al (2000) Interactions of human fibrinogens with factor XIII: roles of calcium and the gamma' peptide. *Biochemistry* 39(22):6698–6705
- Mooney R, Tawil B, Mahoney M (2010) Specific fibrinogen and thrombin concentrations promote neuronal rather than glial growth when primary neural cells are seeded within plasma-derived fibrin gels. *Tissue Eng Part A* 16(5):1607–1619
- Mosesson MW (2005) Fibrinogen and fibrin structure and functions. *J Thromb Haemost* 3(8):1894–1904

- Mosesson MW et al (1972) Human fibrinogen heterogeneities. I. Structural and related studies of plasma fibrinogens which are high solubility catabolic intermediates. *J Biol Chem* 247 (16):5210–5219
- Mosesson MW et al (1989) Identification of covalently linked trimeric and tetrameric D domains in crosslinked fibrin. *Proc Natl Acad Sci USA* 86(4):1113–1117
- Mosesson MW et al (1993) Evidence for a second type of fibril branch point in fibrin polymer networks, the trimolecular junction. *Blood* 82(5):1517–1521
- Mosesson MW et al (1995) The role of fibrinogen D domain intermolecular association sites in the polymerization of fibrin and fibrinogen Tokyo II (gamma 275 Arg→Cys). *J Clin Invest* 96 (2):1053–1058
- Mosesson MW, Siebenlist KR, Meh DA (2001) The structure and biological features of fibrinogen and fibrin. *Ann N Y Acad Sci* 936:11–30
- Moy OJ et al (1988) Fibrin seal adhesive versus nonabsorbable microsuture in peripheral nerve repair. *J Hand Surg Am* 13(2):273–278
- Mozafari R et al (2018) Combination of heterologous fibrin sealant and bioengineered human embryonic stem cells to improve regeneration following autogenous sciatic nerve grafting repair. *J Venom Anim Toxins Incl Trop Dis* 24:11
- Muller-Esterl W (1989) Kininogens, kinins and kinships. *Thromb Haemost* 61(1):2–6
- Muszbek L et al (2011) Novel aspects of factor XIII deficiency. *Curr Opin Hematol* 18(5):366–372
- Mutch NJ et al (2003) Thrombus lysis by uPA, scuPA and tPA is regulated by plasma TAFI. *J Thromb Haemost* 1(9):2000–2007
- Myohanen H, Vaheri A (2004) Regulation and interactions in the activation of cell-associated plasminogen. *Cell Mol Life Sci* 61(22):2840–2858
- Nakayama K et al (2007a) Enhancement of peripheral nerve regeneration using bioabsorbable polymer tubes packed with fibrin gel. *Artif Organs* 31(7):500–508
- Nakayama K et al (2007b) Regeneration of peripheral nerves by bioabsorbable polymer tubes with fibrin gel. *J Nanosci Nanotechnol* 7(3):730–733
- Nesheim M (2003) Thrombin and fibrinolysis. *Chest* 124(3 Suppl):33S–39S
- Ngeow WC (2010) Scar less: a review of methods of scar reduction at sites of peripheral nerve repair. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 109(3):357–366
- Nicoli Aldini N et al (1995) Fibrin sealant conduits in peripheral nerve repair. Springer, Berlin/Heidelberg
- Nishimura MT et al (2008) Mechanical resistance of peripheral nerve repair with biological glue and with conventional suture at different postoperative times. *J Reconstr Microsurg* 24(5):327–332
- Noble J et al (1998) Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. *J Trauma* 45(1):116–122
- Noori A et al (2017) A review of fibrin and fibrin composites for bone tissue engineering. *Int J Nanomedicine* 12:4937–4961
- O'Brien ET 3rd et al (2008) Ultrathin self-assembled fibrin sheets. *Proc Natl Acad Sci USA* 105 (49):19438–19443
- O'Toole TE et al (1990) Affinity modulation of the alpha IIb beta 3 integrin (platelet GPIIb-IIIa) is an intrinsic property of the receptor. *Cell Regul* 1(12):883–893
- Odrlijić TM et al (1996a) Calcium modulates plasmin cleavage of the fibrinogen D fragment gamma chain N-terminus: mapping of monoclonal antibody J88B to a plasmin sensitive domain of the gamma chain. *Biochim Biophys Acta* 1298(1):69–77
- Odrlijić TM et al (1996b) Heparin-binding domain of fibrin mediates its binding to endothelial cells. *Arterioscler Thromb Vasc Biol* 16(12):1544–1551
- Okada M, Blomback B (1983) Calcium and fibrin gel structure. *Thromb Res* 29(3):269–280
- Okumura N et al (2006) A novel variant fibrinogen, deletion of Bbeta111Ser in coiled-coil region, affecting fibrin lateral aggregation. *Clin Chim Acta* 365(1–2):160–167
- Omar MN, Mann KG (1987) Inactivation of factor Va by plasmin. *J Biol Chem* 262(20):9750–9755
- Ornelas L et al (2006a) Fibrin glue: an alternative technique for nerve coaptation – Part I. Wave amplitude, conduction velocity, and plantar-length factors. *J Reconstr Microsurg* 22(2):119–122

- Ornelas L et al (2006b) Fibrin glue: an alternative technique for nerve coaptation – Part II. Nerve regeneration and histomorphometric assessment. *J Reconstr Microsurg* 22(2):123–128
- Owens AP 3rd, Mackman N (2010) Tissue factor and thrombosis: the clot starts here. *Thromb Haemost* 104(3):432–439
- Pandya BV, Cierniewski CS, Budzynski AZ (1985) Conservation of human fibrinogen conformation after cleavage of the B beta chain NH2 terminus. *J Biol Chem* 260(5):2994–3000
- Pertici V et al (2014) Comparison of a collagen membrane versus a fibrin sealant after a peroneal nerve section and repair: a functional and histological study. *Acta Neurochir* 156(8):1577–1590
- Perussi Biscola N et al (2016) Long-standing motor and sensory recovery following acute fibrin sealant based neonatal sciatic nerve repair. *Neural Plast* 2016:9028126
- Pettersson J et al (2010) Biodegradable fibrin conduit promotes long-term regeneration after peripheral nerve injury in adult rats. *J Plast Reconstr Aesthet Surg* 63(11):1893–1899
- Pettersson J et al (2011) Muscle recovery after repair of short and long peripheral nerve gaps using fibrin conduits. *Neurosci Lett* 500(1):41–46
- Pieters M, Wolberg AS (2019) Fibrinogen and fibrin: an illustrated review. *Res Pract Thromb Haemost* 3(2):161–172
- Plow EF, Felez J, Miles LA (1991) Cellular regulation of fibrinolysis. *Thromb Haemost* 66(1):32–36
- Podor TJ et al (2002) Incorporation of vitronectin into fibrin clots. Evidence for a binding interaction between vitronectin and gamma A/gamma' fibrinogen. *J Biol Chem* 277(9):7520–7528
- Povlsen B (1994) A new fibrin seal in primary repair of peripheral nerves. *J Hand Surg Br* 19(1):43–47
- Prydzial EL et al (1999) Plasmin converts factor X from coagulation zymogen to fibrinolysis cofactor. *J Biol Chem* 274(13):8500–8505
- Rafijah G et al (2013) The effects of adjuvant fibrin sealant on the surgical repair of segmental nerve defects in an animal model. *J Hand Surg Am* 38(5):847–855
- Rajangam T, An SS (2013) Fibrinogen and fibrin based micro and nano scaffolds incorporated with drugs, proteins, cells and genes for therapeutic biomedical applications. *Int J Nanomedicine* 8:3641–3662
- Ramos DS et al (2015) Stitchless fibrin glue-aided facial nerve grafting after cerebellopontine angle schwannoma removal: technique and results in 15 cases. *Otol Neurotol* 36(3):498–502
- Raum D et al (1980) Synthesis of human plasminogen by the liver. *Science* 208(4447):1036–1037
- Reichenberger MA et al (2016) ADSCs in a fibrin matrix enhance nerve regeneration after epineural suturing in a rat model. *Microsurgery* 36(6):491–500
- Reinke JM, Sorg H (2012) Wound repair and regeneration. *Eur Surg Res* 49(1):35–43
- Renne T, Gailani D (2007) Role of factor XII in hemostasis and thrombosis: clinical implications. *Expert Rev Cardiovasc Ther* 5(4):733–741
- Rick ME, Krizek DM (1986) Platelets modulate the proteolysis of factor VIII:C protein by plasmin. *Blood* 67(6):1649–1654
- Robinson M, Douglas S, Michelle Willerth S (2017) Mechanically stable fibrin scaffolds promote viability and induce neurite outgrowth in neural aggregates derived from human induced pluripotent stem cells. *Sci Rep* 7(1):6250
- Romano VM et al (1991) Comparison of fibrin glue, bioresorbable tubing and sutures in peripheral nerve repair. *Restor Neurol Neurosci* 3(2):75–80
- Rosso MPO et al (2017) Stimulation of morphofunctional repair of the facial nerve with photobiomodulation, using the end-to-side technique or a new heterologous fibrin sealant. *J Photochem Photobiol B* 175:20–28
- Roth F et al (2017) Platelet-rich fibrin conduits as an alternative to nerve autografts for peripheral nerve repair. *J Reconstr Microsurg* 33(8):549–556
- Ryan EA et al (1999) Structural origins of fibrin clot rheology. *Biophys J* 77(5):2813–2826
- Sahni A, Francis CW (2000) Vascular endothelial growth factor binds to fibrinogen and fibrin and stimulates endothelial cell proliferation. *Blood* 96(12):3772–3778

- Sahni A, Altland OD, Francis CW (2003) FGF-2 but not FGF-1 binds fibrin and supports prolonged endothelial cell growth. *J Thromb Haemost* 1(6):1304–1310
- Sahni A et al (2004) Interleukin-1 β but not IL-1 α binds to fibrinogen and fibrin and has enhanced activity in the bound form. *Blood* 104(2):409–414
- Sakiyama SE, Schense JC, Hubbell JA (1999) Incorporation of heparin-binding peptides into fibrin gels enhances neurite extension: an example of designer matrices in tissue engineering. *FASEB J* 13(15):2214–2224
- Sakiyama-Elbert SE, Hubbell JA (2000a) Controlled release of nerve growth factor from a heparin-containing fibrin-based cell ingrowth matrix. *J Control Release* 69(1):149–158
- Sakiyama-Elbert SE, Hubbell JA (2000b) Development of fibrin derivatives for controlled release of heparin-binding growth factors. *J Control Release* 65(3):389–402
- Sakiyama-Elbert SE, Panitch A, Hubbell JA (2001) Development of growth factor fusion proteins for cell-triggered drug delivery. *FASEB J* 15(7):1300–1302
- Saller MM et al (2018) Validation of a novel animal model for sciatic nerve repair with an adipose-derived stem cell loaded fibrin conduit. *Neural Regen Res* 13(5):854–861
- Salmeron-Gonzalez E et al (2018) Masseter-to-facial nerve transfer: technique and outcomes utilizing a fibrin sealant for coaptation. *J Plast Reconstr Aesthet Surg* 71(8):1216–1230
- Salmeron-Gonzalez E et al (2019) Technical detail on nerve Coaptation in Phalloplasty: use of fibrin glue instead of sutures. *Sex Med Rev* 7(2):376
- Saltz R et al (1991) Experimental and clinical applications of fibrin glue. *Plast Reconstr Surg* 88(6):1005–1015; discussion 1016–7
- Sameem M, Wood TJ, Bain JR (2011) A systematic review on the use of fibrin glue for peripheral nerve repair. *Plast Reconstr Surg* 127(6):2381–2390
- Samis JA et al (2000) Proteolytic processing of human coagulation factor IX by plasmin. *Blood* 95(3):943–951
- Sarhane KA et al (2019) Macroporous nanofiber wraps promote axonal regeneration and functional recovery in nerve repair by limiting fibrosis. *Acta Biomater* 88:332–345
- Scaletta G et al (2019) Obturator nerve regeneration using a genito-femoral graft placed only by fibrin sealant (Tisseel(R)). *Gynecol Oncol* 153(3):703–704
- Schaefer C et al (2014) Pain severity and the economic burden of neuropathic pain in the United States: BEAT neuropathic pain observational study. *Clinicoecon Outcomes Res* 6:483–496
- Schense JC, Hubbell JA (1999) Cross-linking exogenous bifunctional peptides into fibrin gels with factor XIIIa. *Bioconjug Chem* 10(1):75–81
- Schuh CM et al (2015) Activated Schwann cell-like cells on aligned fibrin-poly(lactic-co-glycolic acid) structures: a novel construct for application in peripheral nerve regeneration. *Cells Tissues Organs* 200(5):287–299
- Schuh C et al (2018) An optimized collagen-fibrin blend engineered neural tissue promotes peripheral nerve repair. *Tissue Eng Part A* 24(17–18):1332–1340
- Schwartz ML et al (1973) Human factor XIII from plasma and platelets. Molecular weights, subunit structures, proteolytic activation, and cross-linking of fibrinogen and fibrin. *J Biol Chem* 248(4):1395–1407
- Seddon HJ, Medawar PB (1942) Fibrin suture of human nerves. *Lancet* 240(6204):87–88
- Senses F et al (2016) Effect of platelet-rich fibrin on peripheral nerve regeneration. *J Craniofac Surg* 27(7):1759–1764
- Shainoff JR, Dardik BN (1983) Fibrinopeptide B in fibrin assembly and metabolism: physiologic significance in delayed release of the peptide. *Ann N Y Acad Sci* 408:254–268
- Siconolfi LB, Seeds NW (2001a) Mice lacking tPA, uPA, or plasminogen genes showed delayed functional recovery after sciatic nerve crush. *J Neurosci* 21(12):4348–4355
- Siconolfi LB, Seeds NW (2001b) Induction of the plasminogen activator system accompanies peripheral nerve regeneration after sciatic nerve crush. *J Neurosci* 21(12):4336–4347
- Siconolfi LB, Seeds NW (2003) Mice lacking tissue plasminogen activator and urokinase plasminogen activator genes show attenuated matrix metalloproteases activity after sciatic nerve crush. *J Neurosci Res* 74(3):430–434

- Sidelmann JJ et al (2000) Fibrin clot formation and lysis: basic mechanisms. *Semin Thromb Hemost* 26(6):605–618
- Siebenlist KR, Mosesson MW (1996) Evidence of intramolecular cross-linked A alpha.gamma chain heterodimers in plasma fibrinogen. *Biochemistry* 35(18):5817–5821
- Sierra DH (1993) Fibrin sealant adhesive systems: a review of their chemistry, material properties and clinical applications. *J Biomater Appl* 7(4):309–352
- Silva DN et al (2010) End-to-side nerve repair using fibrin glue in rats. *Acta Cir Bras* 25(2):158–162
- Silva MM et al (2012a) Regulation of fibrinolysis by C-terminal lysines operates through plasminogen and plasmin but not tissue-type plasminogen activator. *J Thromb Haemost* 10(11):2354–2360
- Silva DN et al (2012b) Nerve growth factor with fibrin glue in end-to-side nerve repair in rats. *Acta Cir Bras* 27(4):325–332
- Singer M (1945) The combined use of fibrin film and clot in end-to-end Union of Nerves. *J Neurosurg* 2(2):102
- Sinovskii PV, Filatov AN (1950) Results in application of a fibrin membrane and filaments in neurosurgery; dynamics of absorption of fibrin filaments in the nerve. *Vopr Neurokhir* 14(6):25–29
- Slaughter TF, Greenberg CS (1997) Antifibrinolytic drugs and perioperative hemostasis. *Am J Hematol* 56(1):32–36
- Smahel J, Meyer VE (1995) Fibrin Glueing of peripheral nerves. Springer, Berlin/Heidelberg
- Spartalis E et al (2018) Platelet-rich fibrin and its contradictory effect on peripheral nerve repair after malignant tumor resection: nerve regeneration versus Cancer proliferation. *J Reconstr Microsurg*
- Spotnitz WD (2010) Fibrin sealant: past, present, and future: a brief review. *World J Surg* 34(4):632–634
- Spotnitz WD (2014) Fibrin sealant: the only approved hemostat, sealant, and adhesive—a laboratory and clinical perspective. *ISRN Surg* 2014:203943
- Spotnitz WD, Prabhu R (2005) Fibrin sealant tissue adhesive – review and update. *J Long-Term Eff Med Implants* 15(3):245–270
- Spraggon G, Everse SJ, Doolittle RF (1997) Crystal structures of fragment D from human fibrinogen and its crosslinked counterpart from fibrin. *Nature* 389(6650):455–462
- Stryer L, Cohen C, Langridge R (1963) Axial period of fibrinogen and fibrin. *Nature* 197:793–794
- Suehiro K, Gailit J, Plow EF (1997) Fibrinogen is a ligand for integrin alpha5beta1 on endothelial cells. *J Biol Chem* 272(8):5360–5366
- Sulaiman W, Gordon T (2013) Neurobiology of peripheral nerve injury, regeneration, and functional recovery: from bench top research to bedside application. *Ochsner J* 13(1):100–108
- Tajdaran K et al (2016) An engineered biocompatible drug delivery system enhances nerve regeneration after delayed repair. *J Biomed Mater Res A* 104(2):367–376
- Tarlov IM (1944) Autologous plasma clot suture of nerves: its use in clinical surgery. *JAMA* 126(12):741–748
- Tarlov IM, Benjamin B (1942) Autologous plasma clot suture of nerves. *Science* 95(2462):258
- Tarlov IM, Boernstein W (1948) Nerve regeneration; a comparative experimental study following suture by clot and thread. *J Neurosurg* 5(1):62–83
- Tarlov IM et al (1943) Plasma clot suture of nerves: experimental technic. *JAMA Surg* 47(1):44–58
- Tate KM et al (1987) Functional role of proteolytic cleavage at arginine-275 of human tissue plasminogen activator as assessed by site-directed mutagenesis. *Biochemistry* 26(2):338–343
- Taylor SJ, McDonald JW 3rd, Sakiyama-Elbert SE (2004) Controlled release of neurotrophin-3 from fibrin gels for spinal cord injury. *J Control Release* 98(2):281–294
- Temple CL et al (2004) Resistance to disruption and gapping of peripheral nerve repairs: an in vitro biomechanical assessment of techniques. *J Reconstr Microsurg* 20(8):645–650
- Theberge NP, Ziccardi VB (2016) Use of fibrin glue as an adjunct in the repair of lingual nerve injury: case report. *J Oral Maxillofac Surg* 74(9):1899.e1–1899.e14
- Torul D et al (2018) Comparison of the regenerative effects of platelet-rich fibrin and plasma rich in growth factors on injured peripheral nerve: an experimental study. *J Oral Maxillofac Surg* 76(8):1823.e1–1823.e12

- Travis J, Salvesen GS (1983) Human plasma proteinase inhibitors. *Annu Rev Biochem* 52:655–709
- Trezzini C et al (1988) Fibrinogen association with human monocytes: evidence for constitutive expression of fibrinogen receptors and for involvement of Mac-1 (CD18, CR3) in the binding. *Biochem Biophys Res Commun* 156(1):477–484
- Trezzini C et al (1991) Evidence that exposure to fibrinogen or to antibodies directed against Mac-1 (CD11b/CD18; CR3) modulates human monocyte effector functions. *Br J Haematol* 77(1):16–24
- Tsurupa G et al (2009) Structure, stability, and interaction of the fibrin(ogen) alphaC-domains. *Biochemistry* 48(51):12191–12201
- Undas A, Ariens RA (2011) Fibrin clot structure and function: a role in the pathophysiology of arterial and venous thromboembolic diseases. *Arterioscler Thromb Vasc Biol* 31(12):e88–e99
- van der Hulle T et al (2013a) Variable D-dimer thresholds for diagnosis of clinically suspected acute pulmonary embolism. *J Thromb Haemost* 11(11):1986–1992
- van der Hulle T et al (2013b) Selective D-dimer testing for the diagnosis of acute deep vein thrombosis: a validation study. *J Thromb Haemost* 11(12):2184–2186
- Veklich YI et al (1993) Carboxyl-terminal portions of the alpha chains of fibrinogen and fibrin. Localization by electron microscopy and the effects of isolated alpha C fragments on polymerization. *J Biol Chem* 268(18):13577–13585
- Velada JL et al (2002) Reproducibility of the mechanical properties of Vivostat system patient-derived fibrin sealant. *Biomaterials* 23(10):2249–2254
- Veldman A, Hoffman M, Ehrenforth S (2003) New insights into the coagulation system and implications for new therapeutic options with recombinant factor VIIa. *Curr Med Chem* 10(10):797–811
- Vidigal de Castro M et al (2016) Direct spinal ventral root repair following avulsion: effectiveness of a new heterologous fibrin sealant on Motoneuron survival and regeneration. *Neural Plast* 2016:2932784
- Vieira WF et al (2016) Low-level laser therapy (904 nm) counteracts motor deficit of mice hind limb following skeletal muscle injury caused by snakebite-mimicking intramuscular venom injection. *PLoS One* 11(7)
- von Büngner O (1891) Über die Degenerations- und Regenerationsvorgänge am Nerven nach Verletzungen. *Beiträge zur pathologischen Anatomie* 10:321–387
- Walton BL, Byrnes JR, Wolberg AS (2015) Fibrinogen, red blood cells, and factor XIII in venous thrombosis. *J Thromb Haemost* 13(Suppl 1):S208–S215
- Wang Y et al (2013) A synthetic oxygen carrier in fibrin matrices promotes sciatic nerve regeneration in rats. *Acta Biomater* 9(7):7248–7263
- Wang W et al (2018) Nerve repair with fibrin nerve conduit and modified suture placement. *Anat Rec (Hoboken)* 301(10):1690–1696
- Weisel JW (2004) The mechanical properties of fibrin for basic scientists and clinicians. *Biophys Chem* 112(2–3):267–276
- Weisel JW (2005) Fibrinogen and fibrin. *Adv Protein Chem* 70:247–299
- Weisel JW, Litvinov RI (2013) Mechanisms of fibrin polymerization and clinical implications. *Blood* 121(10):1712–1719
- Weisel JW, Nagaswami C (1992) Computer modeling of fibrin polymerization kinetics correlated with electron microscope and turbidity observations: clot structure and assembly are kinetically controlled. *Biophys J* 63(1):111–128
- Weisshaar CL et al (2011) The potential for salmon fibrin and thrombin to mitigate pain subsequent to cervical nerve root injury. *Biomaterials* 32(36):9738–9746
- Whelan D, Caplice NM, Clover AJ (2014) Fibrin as a delivery system in wound healing tissue engineering applications. *J Control Release* 196:1–8
- Whitlock EL et al (2010) Fibrin glue mitigates the learning curve of microneurosurgical repair. *Microsurgery* 30(3):218–222
- Williams LR (1987) Exogenous fibrin matrix precursors stimulate the temporal progress of nerve regeneration within a silicone chamber. *Neurochem Res* 12(10):851–860

- Williams LR, Varon S (1985) Modification of fibrin matrix formation in situ enhances nerve regeneration in silicone chambers. *J Comp Neurol* 231(2):209–220
- Williams LR et al (1983) Spatial-temporal progress of peripheral nerve regeneration within a silicone chamber: parameters for a bioassay. *J Comp Neurol* 218(4):460–470
- Williams LR et al (1993) Regenerating axons are not required to induce the formation of a Schwann cell cable in a silicone chamber. *Exp Neurol* 120(1):49–59
- Wiman B, Boman L, Collen D (1978) On the kinetics of the reaction between human antipiasmin and a low-molecular-weight form of plasmin. *Eur J Biochem* 87(1):143–146
- Wojtecka-Lukasik E, Maslinski S (1992) Fibronectin and fibrinogen degradation products stimulate PMN-leukocyte and mast cell degranulation. *J Physiol Pharmacol* 43(2):173–181
- Wojtkiewicz DM et al (2015) Social impact of peripheral nerve injuries. *Hand (NY)* 10(2):161–167
- Wood MD et al (2009) Affinity-based release of glial-derived neurotrophic factor from fibrin matrices enhances sciatic nerve regeneration. *Acta Biomater* 5(4):959–968
- Wood MD et al (2013) Fibrin gels containing GDNF microspheres increase axonal regeneration after delayed peripheral nerve repair. *Regen Med* 8(1):27–37
- Wu X et al (2010) Preparation of aligned porous gelatin scaffolds by unidirectional freeze-drying method. *Acta Biomater* 6(3):1167–1177
- Yakovlev S et al (2003) Interaction of fibrin(ogen) with heparin: further characterization and localization of the heparin-binding site. *Biochemistry* 42(25):7709–7716
- Yang Z, Mochalkin I, Doolittle RF (2000) A model of fibrin formation based on crystal structures of fibrinogen and fibrin fragments complexed with synthetic peptides. *Proc Natl Acad Sci U S A* 97(26):14156–14161
- Yang S et al (2001) The design of scaffolds for use in tissue engineering. Part I. Traditional factors. *Tissue Eng* 7(6):679–689
- Yannascoli SM et al (2018) A population-based assessment of depression and anxiety in patients with brachial plexus injuries. *J Hand Surg Am* 43(12):1136.e1–1136.e9
- Yao S et al (2016) Co-effects of matrix low elasticity and aligned topography on stem cell neurogenic differentiation and rapid neurite outgrowth. *Nanoscale* 8(19):10252–10265
- Yin Q et al (2001) Neurotrophin-4 delivered by fibrin glue promotes peripheral nerve regeneration. *Muscle Nerve* 24(3):345–351
- Young JZ, Medawar PB (1940) Fibrin suture of peripheral nerves: measurement of the rate of regeneration. *Lancet* 236(6101):126–128
- Yu P et al (2018) Clinical application of platelet-rich fibrin in plastic and reconstructive surgery: a systematic review. *Aesthet Plast Surg* 42(2):511–519
- Yu Z et al (2020) Application of fibrin-based hydrogels for nerve protection and regeneration after spinal cord injury. *J Biol Eng* 14:22
- Zhao Q et al (1992) The formation of a ‘pseudo-nerve’ in silicone chambers in the absence of regenerating axons. *Brain Res* 592(1–2):106–114
- Zhao Z et al (2014) Improvement in nerve regeneration through a decellularized nerve graft by supplementation with bone marrow stromal cells in fibrin. *Cell Transplant* 23(1):97–110

Silk Biomaterials in Peripheral Nerve Tissue Engineering

Flavia Millesi, Tamara Weiss, and Christine Radtke

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Abstract

Peripheral nerve regeneration represents a major clinical challenge especially if nerve tissue must be replaced to regain function. Repair of critical size nerve defects with development of optimal nerve conduits is the subject of numerous *in vitro* and *in vivo* studies. In this regard, natural silk and silk fibroin are widely used materials

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for tissue engineering of peripheral nerve conduits for implantation. In this chapter, a broad overview of pathophysiological changes and parameters of nerve injuries for repair techniques to achieve nerve regeneration is presented with an emphasis on silk as a biomaterial in tissue engineering. Differences in the variety of silk sources with their individual advantages and disadvantages are discussed with a focus on nerve regeneration. Furthermore, additional components for enhancement of nerve regeneration which must be considered including extracellular matrix proteins, growth factors, peptides sequences, and cellular support to biofunctionalize silk-based nerve conduits are summarized. Finally, clinical translation using experimental *in vivo* models is presented.

1 Introduction

Regenerative medicine's goal is to heal or replace damaged tissues by creating tissue-like implants (Mason and Dunnill 2008). Tissue engineering is the largest subfield of regenerative medicine (Aigner et al. 2018). It uses a combination of cells and biomaterials to design an artificial tissue replacement. Traditional tissue engineering uses a “top-down” approach, in which the components are merely combined and are expected to assemble themselves into structures (Nichol and Khademhosseini 2009). Alternatively, a “bottom-up” approach can be employed. Here, structural tissue-like features are designed by assembling building units (Elbert 2011), and the choice of material depends on the intended application.

The ideal material for nerve tissue engineering requires the combination of stability and elasticity (Moore et al. 2009). Furthermore, it should have appropriate permeability and allow cell growth (Cao and Wang 2009). Most importantly, it needs to be nonimmunogenic, biocompatible, and biodegradable (Lloyd 2002).

Both synthetic and biological materials have found their use in regenerative medicine (Seal et al. 2001). However, synthetic materials often lack elasticity and have poor integration (Lally et al. 1993; Schafer-Nolte et al. 2014). In addition, they have been linked to fibrosis and rigidity which leads to pain and discomfort (Brown and Finch 2010). In turn, biomaterials like collagen, gelatin, and chitosan show superior biocompatibility, biodegradability, and bioresorbability (Evans et al. 2002), but they often come hand in hand with problems like high variation and low quantity (Aigner et al. 2018). Additionally, they are potentially highly immunogenic and can degrade too fast when used *in vivo*. Biomaterials also include silk fibroin fibers, which impress with high tensile strength and elasticity compared to commonly used natural materials (Liu et al. 2008). They demonstrated slow biodegradation and noncytotoxic characteristics (Horan et al. 2005; Gellynck et al. 2008). Moreover, they are noninflammatory and have shown low antigenicity (Zeplin et al. 2014). There are two major silk producing species that have been investigated thoroughly over the past few decades: the silkworm *Bombyx mori* and the spider genus *Nephila* (Altman et al. 2003). Silk fibers of both species have been used for medical purposes and their advantages and disadvantages as well as their application in nerve regeneration will be discussed in this chapter.

2 Peripheral Nerve Injuries

Peripheral nerve injury results in degeneration of axons distal to the site of insult and a functional deficit of the affected extremity. In contrast to the central nervous system, the peripheral nervous system (PNS) possesses a regenerative ability, which is primarily ascribed to the inherent plasticity of Schwann cells. This plasticity enables an adaptive response of Schwann cells to the degenerating axons by dramatic gene expression changes that results in the generation of a specialized repair cell (Arthur-Farraj et al. 2012; Jessen et al. 2015). Repair Schwann cells exert novel functions essential for nerve regeneration that comprise myelin clearance, immune regulation, as well as neurotogenic and neurotrophic support (Jessen and Mirsky 2016). Hence, nerve defects obtained by contusion or crush and even nerve gaps less than 5 mm usually regenerate spontaneously (Johnson et al. 2005). In contrast, severe nerve damage involving tissue destruction or loss substantially harms the nerve integrity and entails a short- or long-distance nerve gap. The regrowing axons face the challenge to overcome the nerve gap and reach the distal nerve segment before scar tissue infiltration or chronic denervation occurs. Prolonged lack of axonal contact was shown to diminish the regenerative support of Schwann cells, which is considered as one of the main reasons for progressive regeneration failure in humans (Hoke 2006; Sulaiman and Gordon 2009). In addition, the loss of nerve structure and guiding cues can lead to disorganized sprouting of regenerating axons, neuroma formation, and pain (Daroff et al. 2016). Thus, time is a critical factor for successful regeneration and functional recovery of severely injured peripheral nerves (Ray and Mackinnon 2010).

3 Strategies for Peripheral Nerve Repair

3.1 Surgical Intervention

Reconstructive surgery has emerged as an important tool in the treatment of peripheral nerve injuries and different procedures have been established dependent on the underlying nerve defect (Ray and Mackinnon 2010). In case of nerve transection, nerve endings may be reconnected when secure and tension free end-to-end nerve coaptation is possible (Millesi and Schmidhammer 2007). To avoid denervation and fibrosis of distal motor end plates after injury, the motor end plate can also be “babysitted” by end-to-side nerve coaptation to a donor nerve until the axons reinnervate the target muscle (Haastert et al. 2010; Gyori et al. 2018). But this procedure inflicts damage on the donor nerve and may impair its function while providing only a variable amount of regenerative success in the recipient nerve (Millesi and Schmidhammer 2007).

3.2 Nerve Grafts

The current gold standard to replace lost nerve tissue is the transplantation of an autologous nerve graft (Ray and Mackinnon 2010). Nerve autografts are immunologically compatible and provide the benefits of an existing nerve architecture and

Schwann cell support. However, the availability of expendable donor nerve tissue is limited and comes with the sacrifice of a functional, mostly sensory, nerve causing sensory loss, donor site morbidity, and possible neuroma formation (Grinsell and Keating 2014; Kanno et al. 2015).

Nerve allografts offer a readily available alternative and allow matching of the donor nerve graft to the damaged nerve concerning motor/sensory specificity, size, and location (Moore et al. 2011). In order to reduce allograft immunogenicity, different processing techniques have been established to remove cellular components but preserve as much of the inherent nerve structure as possible (Crapo et al. 2011). Recent studies support the use of acellular nerve allografts for surgical management of small- and large-diameter nerve defects in rat models but also point out that the processing technique affected the axonal regeneration success (Whitlock et al. 2009; Moore et al. 2011; Hundepool et al. 2017). Still, acellular nerve allografts are inferior to autografts, presumably due to the lack of Schwann cells or other cells involved in nerve regeneration and the degradation of extra cellular matrix components and growth factors during the processing procedures (Jiang et al. 2010; Hundepool et al. 2017).

3.3 Artificial Nerve Conduits

A tempting approach for nerve reconstruction is the implementation of artificial nerve conduits. The ideal conduit shall provide structural and physiological support that mimics the nerve environment. Tissue engineering represents a promising research field for the reconstruction of damaged tissue by combining engineering and life science strategies. A major advantage represents the possibility of a tailored conduit design to match the implantation site. Artificially engineered nerve conduits should offer a suitable guiding structure, protection of regenerating axons from scar tissue infiltration, and easy handling. In addition, the selection of an appropriate material must take into account intrinsic material properties such as degradation characteristics and tissue compatibility (Muheremu and Ao 2015). Artificial nerve conduits should possess flexibility but also provide mechanical resistance for suture stability. Another important property is the conduit's permeability to enable the supply with nutrients and oxygen at the site of regeneration. Also swelling properties of the used material must be carefully assessed because a reduction of the conduit's inner diameter will lead to nerve compression and pain. As a consequence, nerve conduits made of different synthetic or biological materials have been developed (Arslantunali et al. 2014). Many of them induced regenerative effects comparable to nerve autografts, especially for short distance nerve defects, in previous studies (Schlosshauer et al. 2006; Jiang et al. 2010). However, the therapeutic effect of artificial nerve conduits toward long nerve defects remains unsatisfactory (Moore et al. 2009; Jiang et al. 2010). Hence, novel methods and materials are needed to develop further treatment options for long nerve defects in clinical practice.

4 Advantages of Silks as Biomaterial in Tissue Engineering

Both synthetic and biological materials have found their use in tissue engineering (Seal et al. 2001). Synthetic materials are easily produced and their mechanical properties can be controlled to meet the need of the transplantation site. However, synthetic polymers often lack elasticity, poorly integrate into the native tissue, and have been linked to inflammation and fibrosis causing pain and discomfort (Lally et al. 1993; Brown and Finch 2010; Schafer-Nolte et al. 2014). This is why research intensively investigates biomaterials like collagen, gelatin, and chitosan for tissue reconstruction. These natural polymers provide biological binding sites for cells and show superior biocompatibility, biodegradability, and bioresorbability (Evans et al. 2002). Still, their relative expense, degradation kinetics, and limited mechanical properties impede their clinical use (Aigner et al. 2018).

Compared to other natural materials, silk is a promising biopolymer for biomedical applications as it combines high tensile strength and elasticity (Liu et al. 2008). There are two major silk-producing species that have been investigated thoroughly over the past few decades: the silkworm *Bombyx mori* and the spider genus *Nephila* (Altman et al. 2003). Silk fibers of both species have been used for medical purposes and their advantages and disadvantages are discussed in the following.

4.1 Silk

Silk is composed of fibrous proteins and its production is unique to three out of the four arthropod classes: millipedes (Myriapoda), insects (Insecta), and spiders (Arachnida) (Craig 1997; Qi et al. 2017). They secrete silk by creating a water-insoluble solid fiber from a viscous concentrated protein solution (Lefevre et al. 2011). Silk biopolymers are primarily composed of β -sheet crystallites, α -helices, and coils, and the unique composition of these entities gives fibers an extraordinary strength (Sutherland et al. 2010). Although silk from insects and spiders show astounding resemblances (Gatesy et al. 2001), silk secretion evolved independently in both groups (Craig 1997; Sutherland et al. 2010). Differences lie in the molecular structure of the silk proteins such as their amino acid sequence and structural conformation (Gage and Manning 1980; Mita et al. 1994; Hayashi and Lewis 2000).

4.1.1 Silk from Insects

The silk production of all insects is limited to a certain period of an insect's life, the larvae stage (Craig 1997; Sutherland et al. 2010). The fibrous silk proteins are generated in Malpighian tubules, dermal glands, or modified salivary glands, i.e., the labial glands (Sehnal and Akai 1990; Craig 1997; Aigner et al. 2018). One of the most important examples for silk producing insects is the domestic silkworm *Bombyx mori* (Goldsmith et al. 2005; Xia et al. 2014). Silkworm-derived silk fibers have already been used 5000 years ago in China. Their story started when a silkworm cocoon fell from a mulberry tree into princess Xi Ling Shi's cup of tea,

which caused the unraveling of the cocoon into individual fiber threads (Soumya et al. 2017). Since then, the silk fibers found their way into the textile industry and were also used for medical applications such as suture material and wound dressings (Kurioka and Yamazaki 2002; Cao and Wang 2009).

Bombyx mori-secreted cocoon silk is composed of two major proteins, fibroin (60–80%) and sericin 15–35% (Cao and Zhang 2016). Other components (1–5%) include wax, pigments, and sugar. Sericin is a glue-like protein that binds two fibroin fibers together when the silk is spun (Mondal et al. 2006) and aids pupa development and metamorphosis by protecting the larvae against changing weather conditions (Jena et al. 2018). The fibrous fibroin consist of mainly two nonpolar amino acids, glycine and alanine (Inoue et al. 2003; Yang et al. 2004; Chen et al. 2016), whereas sericin contains mostly polar amino acids (Zhang 2002; Padamwar and Pawar 2004). Of note, the silk fibers were reported to elicit an inflammatory response *in vivo*, which is presumably triggered by sericin (Soong and Kenyon 1984; Altman et al. 2003). To avoid this problem, several methods of removing the hydrophilic sericin components from the fibroin fibers have been established (Freddi et al. 2003; Cao and Zhang 2016). The outermost sericin layer, which surrounds the fibroin fiber, can be dissolved by cooking the silk cocoons in hot water, while the innermost sericin is only dissolved in boiling alkaline water (Altman et al. 2003). This thermochemical process is referred to as degumming or refining. However, it has been shown that remnants of sericin remain which may cause difficulties in clinical translation of *Bombyx mori* silk. Additionally, the drastic method of removing sericin can affect the microstructure and therefore the mechanical properties of the fibroin silk fibers (Pérez-Rigueiro et al. 2001; Pérez-Rigueiro et al. 2002; Freddi et al. 2003; Teh et al. 2010).

In summary, silkworm-derived silk is biodegradable and shows a considerable mechanical strength (Altman et al. 2003; Qi et al. 2017). The big advantage of the *Bombyx mori* larvae is their easy keeping in high numbers for silk farming in so-called sericultures (Sun et al. 2012). Additionally, their silk can be harvested in large quantities from their cocoons each containing up to 1300 meters of silk fibers (Xia et al. 2014). However, processing procedures for sericin removal are necessary prior to their medical application, which affect their mechanical properties.

4.1.2 Silk from Spiders

The taxonomical class name Arachnida for spiders descend from Ovid's *Metamorphoses*, a single poem of 15 books, including the myth of Arachne, a talented weaver, and Athena. Not only was Arachne's art of weaving far more beautiful than any of Athena's work, her woven cloths also depicted ways in which the gods had failed and abused humans. Athena was enraged and turned Arachne into a spider condemned to continue weaving forever (Ovid and Humphries 1983). Similar to silkworm-derived silk, the potential of spider silk fibers for medical applications was discovered in ancient Greece and Rome (Newman and Newman 1995). In some cultures, spider silk served as fishing lines (Heim et al. 2009) and later, silk fibers also found their use in the military (Gerritsen 2002).

Female araneoid spiders produce four types of silk fibers and three types of protein glue within seven different glands in their abdomen (Kovoor 1987; Craig 1997). The glands end in the so-called spigots on top of the spinnerets through which silk proteins are assembled and secreted (Fig. 1) (Foelix 1996). Due to the variety in glands, spiders are capable to produce more than one silk simultaneously (Foelix 1996). Spider silk proteins consist of repetitive amino acid sequences (Rising et al. 2011). There are four amino acid motifs, which are unique to the major ampullate gland and the flagelliform gland: An, GA, GGX, and GPG(X)_n. In addition, silk contains crystalline and noncrystalline components (Rising et al. 2011). The crystalline and noncrystalline fractions can vary between different silks and can also change under specific conditions like hydration (Gosline et al. 1999). For most experiments, the silk from the major ampullate gland is used, which is called dragline silk (Knight and Vollrath 2001). A spider typically uses these fibers for the strong outer frame of the net as well as a lifeline for escaping danger (Rhisiart and Vollrath 1994; Vollrath and Porter 2006). Spider dragline silk of *Nephila* contains two different kinds of proteins that vary in their amino acid content: Spidroin 1 (MaSp1) and Spidroin 2 (MaSp2) (Brooks et al. 2008). MaSp1 is associated with the silk's tensile strength and composed of repeated motifs of mostly alanine and glycine (Hinman and Lewis 1992; Xu et al. 2007). In addition to these motifs, MaSp2 also contains 15–17% proline (Gosline et al. 1999), which is suggested to cause the silk's elasticity as it inhibits the formation of crystalline structures (Thiel et al. 1997; Hayashi and Lewis 2000).

The application of spider silk has mostly been studied in the spider genus *Nephila* (Fig. 1) (Vollrath 1992; Vollrath 1993; Vollrath 2000; Knight and Vollrath 2001;

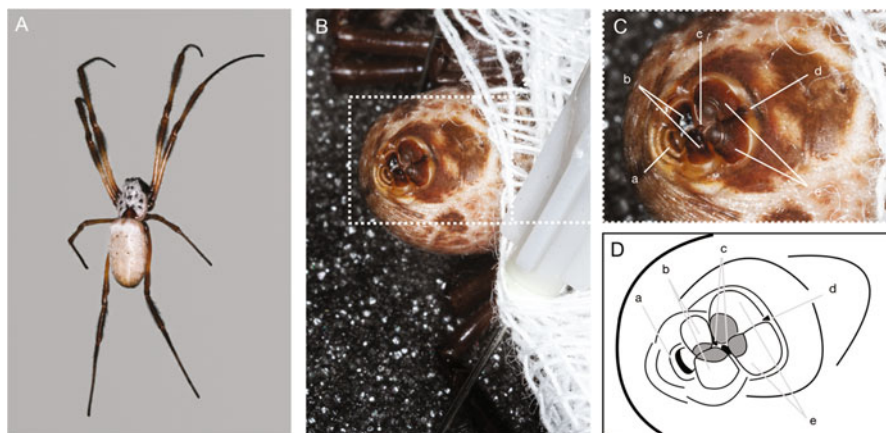


Fig. 1 Individual *Nephila edulis* and her abdomen with visible spinning organs. (a) Adult female *Nephila edulis* in her web. (b) Abdomen of a fixed spider. (c) Close up and (d) schema of the abdomen of a female *Nephila edulis* with spinnerets through which silk and protein glue are secreted, as well as anus (a) and colulus (d). Anterior (e) and posterior (b) spinnerets are visible, whereas the medial spinnerets (c) lie underneath and in between the other spinnerets. The colulus (d) has no known function (Foelix 1996) and is reduced in many other spider families

Vollrath 2005; Vepari and Kaplan 2007). The name *Nephila* is Ancient Greek and means “fond of spinning” (Cameron 2005). The harvest of spider silk can be easily performed by fixing the spider and pulling the silk fiber directly out of the spinneret, the silk-spinning organ (Madsen et al. 1999). Silk harvesting can be repeated on a daily basis but compared to the silkworm-derived cocoon silk, the yield per animal is lower (Aigner et al. 2018). Notably, the quality of the silk fibers depends on multiple variables such as the species of spider, the individual spider, and its keeping as well as the harvesting process (Pérez-Rigueiro et al. 2005). In addition, adult female spiders are often territorial and cannibalistic which increases the needed space per animal (Scheibel 2004; Yip and Rayor 2014). Another major difference of spiders is their continuous production of silk throughout their lives, whereas silk production in insects is limited to the larvae stage (Craig 1997, #21). A further advantage over other biomaterials is the outstanding plasticity of spider silk fibers (Work 1976; Vollrath 1993; Vollrath 2000). Adult araneoid spiders are able to adapt the MaSp1 and MaSp2 protein composition of silk fibers according to environmental factors as well as to control the fiber diameter and rate at which the fiber is drawn (Craig 1997; Craig et al. 1999). Thus, spider silk fibers impress with tunable mechanical properties such as elasticity and strength (Liu et al. 2008; Blamires et al. 2017). Spider silk is considerably tougher than man-made synthetic fibers, e.g., Kevlar 49, and five times tougher than steel by weight (Heim et al. 2009). It has been shown that manufactured spider silk sutures have a higher stress to strain ratio and a greater tensile strength than nylon sutures (Kuhbier et al. 2011). Additionally, spider silk fibers can withhold temperatures up to 230 °C (Cunniff et al. 1994) and have an incredible tensile strength up to 1.1 GPa and breaking energy of up to 520 MJ/m³ (Madsen et al. 1999; Agnarsson et al. 2010). Furthermore, spider silk is long-term degradable which is of special interest for medical approaches (Sponner et al. 2005). An important difference to the silk of *Bombyx mori* is that native spider silk fibers are nonimmunogenic *in vivo* as they do not contain sericin (Allmeling et al. 2008, Kuhbier et al. 2011, Radtke et al. 2011).

Hence, spider silk fibers may show high variations and cannot be harvested as intensively as silkworm-derived silk, but they impress with extraordinary mechanical properties. Moreover, they are long-term biodegradable and immunologically tolerated, which is advantageous for clinical translation.

4.1.3 Artificially Produced Silk

Scientists over the past few decades came to two conclusions: Firstly, silk from insects and spiders are superior to other biomaterials (Schafer-Nolte et al. 2014). Collagen, for example, is cheap and abundant, but linked to impurities, disease transmission, and risk for bacterial infections (Ramshaw et al. 2014, #91). Secondly, silk from spiders is superior to silkworm-derived silk in regard to its mechanical properties and risk of inflammation *in vivo* (Vollrath 2005; Allmeling et al. 2008). Nevertheless, one major disadvantage remains, namely that spider silk fibers are less accessible than other biomaterials and cannot be harvested in high quantities necessary for commercial use (Aigner et al. 2018). Therefore, new methods have been

developed to produce large amounts of spidroin that is consistent and biologically safe (Heidebrecht and Scheibel 2013).

New insights in genetics gave rise to new possibilities by genome editing and recombinant DNA (Heidebrecht and Scheibel 2013; Aigner et al. 2018). Gene editing describes the method of modifying, deleting, replacing, and/or inserting DNA into the genome of a living organism (El-Bassyouni and Mohammed 2018). Hereby, transgenic animals can be created, an organism whose genome has been edited through recombinant DNA technology (Goldman et al. 2004). This involves either the combining of DNA from different organisms or the insertion of foreign DNA into the genome (Maksimenko et al. 2013, #54). Spider silk fibers are the ideal biomaterial, but they cannot be produced on a large scale. This could be overcome by the recombinant production of natural spidroins and/or their essential features (Heidebrecht and Scheibel 2013, #41). Different host species have been used such as bacteria, yeast, insect cells, plants, mammalian cells, and transgenic mammals. Bacteria like *Escherichia coli* (*E. coli*) are advantageous because of their simplicity and well-known genetics (Sorensen and Mortensen 2005). Moreover, they are easy and cheap to keep and allow fast results due to their short life cycle. When used to produce silk spidroin, *E. coli* demonstrated promising results in a wide range of applications from micrometer-fibers and nanofibers over films, foams, and hydrogels (Aigner et al. 2018). On the other hand, the production of silk fibers in *E. coli* has also shown limitations such as translation limits (Rising et al. 2011), depletion of rRNA (Rosenberg et al. 1993), low protein yield (Arcidiacono et al. 1998), heterologous proteins (Xia et al. 2010), and low protein solubility (Bini et al. 2006). Furthermore, *E. coli* cannot perform eukaryotic posttranslational modifications which results in incorrect protein-folding and consequently, in underdeveloped cell growth (Kukuruzinska and Lennon 1998, #49). Another host species for recombinant DNA technology is yeast (Heidebrecht and Scheibel 2013). In contrast to *E. coli*, yeast can express high molecular weight proteins like spidroin (Cregg 2007). These proteins were able to be processed into meshes and films as well as fibers, but their mechanical properties did not rival that of native spider silk (Bogush et al. 2009). Spider silk fibers have also been produced in transgenic plants, which demonstrated advantages such as low cost (Barr et al. 2004, #13) and high protein yields (Moloney and Holbrook 1997; Conrad and Fiedler 1998), but have disadvantages like complicated genetic manipulation and longer generation intervals (Heim et al. 2009; Heidebrecht and Scheibel 2013). Lastly, transgenic animals have also been used for the production of recombinant spider silk proteins. In transgenic goats and mice, the proteins can be secreted with milk, which results in high protein yields (Heim et al. 2009). But the creation of transgenic mammals is expensive and time consuming (Xu et al. 2007). Transgenic *Bombyx mori* can also be used to produce spider silk spidroin fibers (Xu et al. 2018), which would combine the spider silk's properties with the advantageous handling and harvesting of *Bombyx mori*. These experiments resulted in superior silk to silkworm-derived fibers, but the fibers did not rival the native spider silk fibers (Wen et al. 2010, #109).

To summarize, no method of recombinant DNA technology achieved to stand out since all of them have advantages and disadvantages. But it is important to note that this kind of research is still in its earlier phases and future studies may bring new outlooks.

4.1.4 Silk Modification

Silk from both insects and spiders has been discovered as a material that can be manufactured and manipulated (Altman et al. 2003). The simplest modifications to silk fibers are the physical adsorption or chemical immobilization of proteins to provide surface interactions for cells (Vepari and Kaplan 2007).

Another modification method is to directly extract fibroin from dissolved fibers of *Bombyx mori* (Mandal and Kundu 2008; Heidebrecht and Scheibel 2013; Aigner et al. 2018). Subsequently, various different solid forms can be created from the silk protein solution. For tissue regeneration, the regenerated silk fibroin can be formed into ropes, braided and textured yarns, as well as into a knitted silk structure (Altman et al. 2003). Moreover, the artificial silk dopes can be spun using a range of techniques from wet-spinning to electrospinning (Aigner et al. 2018). Wet-spinning describes fiber formation in a coagulation bath by protein precipitation (Matsumoto et al. 1996). Electrospinning refers to the use of an electrical field in order to evaporate the solvent by accelerating the spinning solution (Zarkoob et al. 2004). The remade fibers can be further modified by creating silk fibroin mats, films, hydrogels, and porous sponges (Vepari and Kaplan 2007). Unfortunately, the remade fibers are inferior to natural silk fibers concerning their mechanical stability (Madsen et al. 1999; Xie et al. 2006) and structural integrity (Putthanarat et al. 2000). Moreover, because of the exposure to high temperatures, the protein conformation (Zuo et al. 2006, #143) and the molecular weight of the proteins are altered (Iridag and Kazanci 2006).

In conclusion, silk from both *Bombyx mori* and the spider genus *Nephila* have demonstrated to be suitable materials for nerve regeneration. *Bombyx mori* can be easily harvested, but the protein sericin causes inflammation *in vivo*. Spider silk fibers cannot be harvested in high quantities, but show outstanding mechanical properties and are nonimmunogenic *in vivo*. Different methods have been established to further enhance the silk fibers' properties. Genome sequencing and gene editing gave rise to new opportunities by creating spidroin silk fibers via recombinant DNA technology and transgenic organisms. Although recombinant silk fibers have already been established for cosmetic purposes and among others to produce a \$314 tie made entirely of spider silk, its use in medicine, especially nerve regeneration, is still relatively little researched (DeFrancesco 2017). This chapter further discusses the already published applications of silk biomaterials from *Bombyx mori* silk and *Nephila* silk for application in nerve regeneration *in vitro* and *in vivo*.

5 Silk as Biomaterial in Peripheral Nerve Repair

A growing body of studies has been investigating silk obtained from different species as novel biomaterial for biomedical applications (Kundu et al. 2013; Tokareva et al. 2014; Melke et al. 2016; Radtke 2016; Wu et al. 2017; Magaz et al. 2018). In the following, the dragline silk of the *Nephila* spider as well as processed cocoon silk from the silkworm *Bombyx mori* in different approaches for

peripheral nerve regeneration will be emphasized. The tested applications comprise (1) silk fibers, especially as conduit filling material and guiding structure, and (2) regenerated silk fibroin for nerve graft construction, with or without additional biological modifications (biofunctionalization).

5.1 Biocompatibility of Silk with Neural Cells

In order to proof the biocompatibility of silk for peripheral nerve repair, several studies evaluated the response of peripheral nerve cells to silk fibers *in vitro*. Yumin Yang et al. cultured rat dorsal root ganglia (DRG) on 0.7–0.8 cm long degummed silk fibers from the silkworm *Bombyx mori* and observed Schwann cell outgrowth and neurite extension along the fibers. In addition, a silk fibroin extraction fluid, consisting of 100 mg fibroin per 1 ml medium, did not affect morphology, viability, or proliferation of isolated rat Schwann cell cultures (Yang et al. 2007a). In a subsequent study, it was shown that degummed silk fibers also allowed adherence and neurite outgrowth of hippocampal neurons. Again, the silk fibroin extract had no effect on morphology, marker expression, or viability of hippocampal neurons (Tang et al. 2009).

In contrast to *Bombyx mori* cocoon silk, the native dragline silk of spiders lacks the sericin protein, which omits the need for a degumming procedure. Hence, the superior mechanical properties of spider silk concerning strength and flexibility are preserved and the dragline silk can be readily used for experimentation after sterilization. The first study that addressed the biocompatibility of native spider silk fibers with Schwann cells was published by Allmeling et al. They reported a quick adherence of Schwann cells to the fibers of the genus *Nephila* followed by migration and proliferation along the fibers in a dense and longitudinal fashion (Allmeling et al. 2006). Roloff et al. elegantly visualized the behavior of neuronal NT2 cells in response to *Nephila* spider silk fibers, which revealed neuronal differentiation into ganglion-like structures interconnected by bundled neurites aligned along the fibers (Roloff et al. 2014). Our group adapted the method by Allmeling et al. to comprehensively investigate the biological behavior of Schwann cells in response to spider silk fibers (see Fig. 2).

5.2 Silk-Based Nerve Grafts in Nerve Regeneration

Most reports that address silk-based materials for the development of nerve conduits involve silk derived from the *Bombyx mori* silkworm due to its easy harvesting and versatile processing opportunities (Kundu et al. 2013). The cocoon silk fibers can be twisted into yarn, partially solubilized, or completely dissolved into an aqueous fibroin solution. Further processing of the regenerated silk fibroin allows to produce basically four scaffold forms: mats, sponges, hydrogels, and films with adjustable properties to meet the demands of the final application (Rockwood et al. 2011). Different combinations and modification of these materials were used in many biomedical applications and an overview of strategies for nerve conduit development is given below.

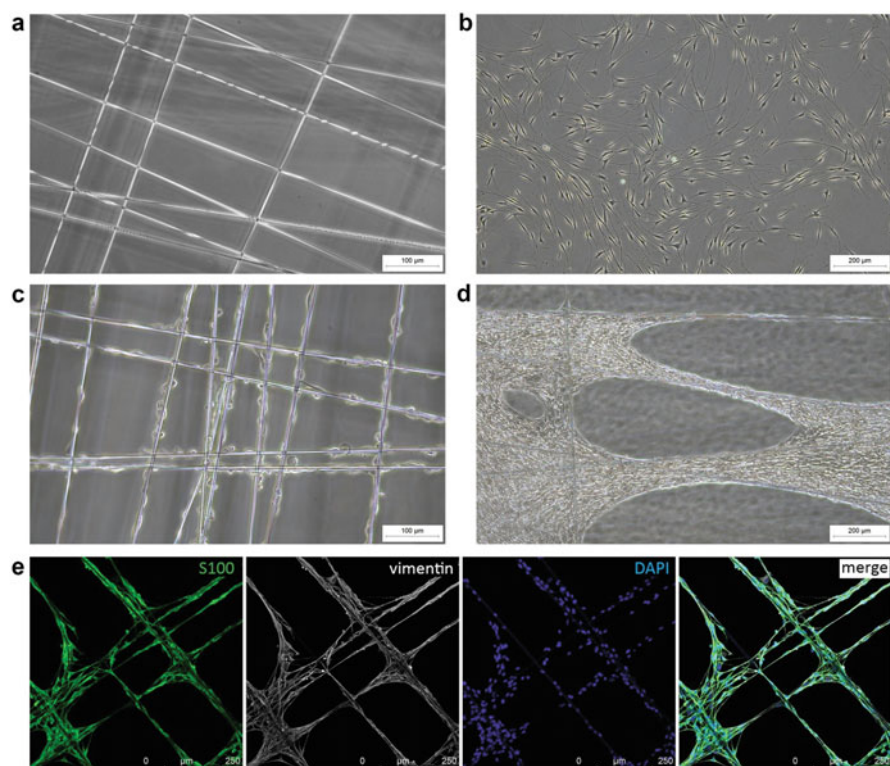


Fig. 2 Schwann cell cultures on spider silk. (a) Phase contrast image of *Nephila* dragline spider silk fibers cross reeled around a handcrafted stainless steel frame. (b) Phase contrast image of a rat Schwann cell culture grown on Poly-L-Lysine/laminin coated wells. Phase contrast images of rat Schwann cells grown on the spider silk after (c) 5 days and (d) 25 days. (e) Immunofluorescence image of rat Schwann cells grown on spider silk fibers for 20 days stained for Schwann cell marker S100 (green), intermediate filament vimentin (white), and DAPI (blue)

5.2.1 Silk-Based Conduits

The ideal artificial nerve conduit shall exhibit a three-dimensional network that mimics structural and biochemical features of the extracellular matrix (ECM) to provide optimal support for cell survival, migration, and proliferation. Thus, silk-fibroin-based structures as well as braided silk have been investigated as source material for nerve conduit development (Fig. 3).

The basic conduit structure, a hollow tube of varying diameter, can be prepared from a fibroin solution by molding, dip-coating, and gel- or electrospinning around a rotating mandrel (Lovett et al. 2008; Rockwood et al. 2011). In addition, a textile-engineered raw silk conduit derived from twisted *Bombyx mori* silk yarn was reported by Teuschl et al. (2015). Injection molding or dip-coating of stainless steel wires results in tubular structures with random fiber deposition. Since the ECM is usually composed of organized fibers, structural alignment of fibroin is

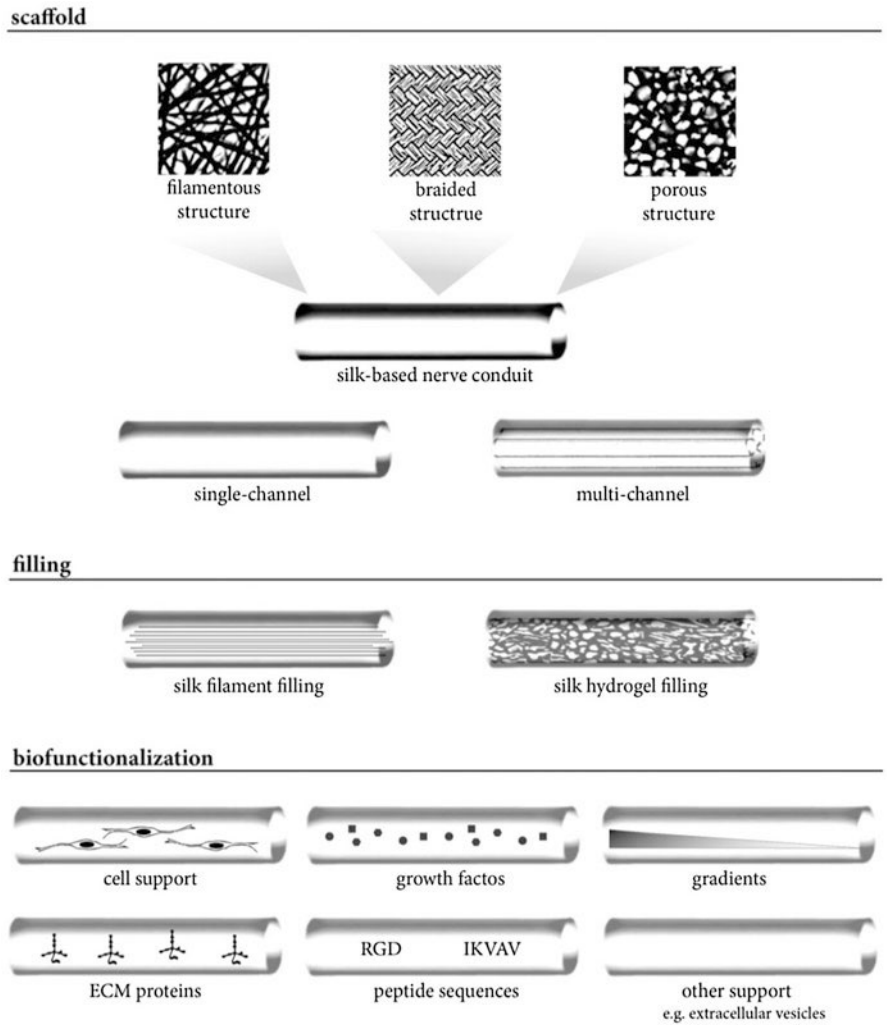


Fig. 3 Schematic overview of fibroin-based nerve conduits

considered more appropriate for conduit construction. Aligned fibers from aqueous fibroin can be produced via gel- or electrospinning. Gel-spinning generates fibers through the shear forces applied by a small gauge needle, while electrospinning uses a strong electric field that reduces the fiber diameter. Another strategy is the production of silk-based nonwoven fibrous mats from partially solubilized silk fibers or by electrospinning of aqueous fibroin solution on a plane area that have to be rolled up to form a three-dimensional scaffold. The manufactured conduits can then be subjected to various processing steps, essentially involving air-drying, lyophilization, solvent and/or alcohol treatment, which influence the final surface properties

such as porosity and compressive strength (Nazarov et al. 2004; Vepari and Kaplan 2007). The mechanical features of the scaffold are important for optimal cell behavior and should provide topographical cues to support directed regrowth of the proximal nerve ending. For example, Zhang et al. managed to achieve control over the cross-section morphology of scaffold channels by adapting the freezing procedure of aqueous fibroin. This way, 3D scaffolds with parallel pores were obtained that formed uniaxial multichannel with a 42–142 μm diameter. The inner surface of the channels also contained numerous parallel aligned ridges, which provided continuous contact guidance for outgrowing primary hippocampal neurons (Zhang et al. 2012).

Fibrous mats generated by electrospinning are highly suitable for the attachment and growth of cells by providing porosity and high surface area-to-volume ratio (Min et al. 2004; Unger et al. 2004). Indeed, Schwann cells cultured on an interconnected porous network consisting of electrospun fibroin nanofibers of 221–389 nm diameters showed similar proliferation and growth factor secretion as compared to polylysine substrate controls (Hu et al. 2012). Electrospinning further allows to tailor structural parameters by adjusting the nanofiber diameter, orientation, and size (Zhang et al. 2009). Importantly, the nanofiber diameter of electrospun structures was shown to affect cellular behavior such as migration and neurite outgrowth (Wang et al. 2010; Qu et al. 2013). Madduri et al. used electrospinning for the deposition of aligned silk fibroin nanofibers of 400–500 nm diameters onto fibrous silk mats. In contrast to randomly arranged nanofibers, the aligned fibers facilitated migration and orientation of Schwann cells and promoted unidirectional guidance for sensory as well as motor axons derived from chick embryos (Madduri et al. 2010). Hence, modifying the inner luminal wall of fibrous conduits with aligned fibers is assumed to create a physical guiding structure that improves axonal pathfinding and target reinnervation. Proof of principle was provided by an electrospun silk fibroin tube developed by Hu et al. The conduit featured a bilayer structure with an inner layer of aligned nanofibers as guiding cues and an outer layer with randomly arranged nanofibers for stabilization. Three months after implanting the conduit to bridge a 5-mm long rat facial nerve defect, nerve regeneration was similar to nerve autograft controls (Hu et al. 2013). In another approach, Dinis et al. created electrospun mats of aligned nanofibers with an average diameter of 792 nm. Subsequently, the mats were rolled around 0.3 mm diameter Teflon[®] sticks to form conduits with longitudinally oriented multichannels that mimicked a perineurium fascicular architecture and mechanical properties of a rat sciatic nerve (Dinis et al. 2015).

5.3 Filling Material for Nerve Conduits

5.3.1 Natural Silk Fibers

Artificial nerve conduits containing an internal framework as guiding structures are suggested to enhance directed axonal outgrowth by inhibiting disorganized axon sprouting (Daroff et al. 2016). Given their supportive cellular guiding properties *in vitro*, native spider silk was tested as filling material for artificial nerve conduits

consisting of decellularized veins. Allmeling et al. supplemented the spider-silk-lined vein conduit with human Schwann cells dissolved in matrigel and observed that the Schwann cells completely enclosed the fibers and formed columns similar to “Bands of Bügner” after 7 days *in vitro* culture. In a next step, this nerve conduit was tested in a 20 mm sciatic nerve defect in rats. After 6 months, vein conduits containing spider silk showed significantly increased regeneration when compared to the matrigel-containing controls (Allmeling et al. 2008). Based on these findings, the setting was implemented in a long distance (> 5 cm) tibial nerve injury model in adult sheep by Radtke et al. (2011). Notably, the results demonstrated that there was no significant difference in the amount of regenerated axons and electrophysiological recordings between the autologous nerve grafts and decellularized pig venules filled with spider silk after 6 and 10 month post-surgery. Despite the promising results of spider silk filled conduits in animal studies, no conduits are available for nerve defects exceeding 10 cm. Hence, the experimental setting of Allmeling et al. was modified to construct conduits consisting of decellularized ovine vessels and *Nephila edulis* dragline silk for 4 cm, 10 cm, and 15 cm long nerve defects (Kornfeld et al. 2016). *In vitro* analysis of Schwann cell behavior within the conduits successfully demonstrated survival, proliferation, and migration throughout the conduits. Taken together, the findings underline that spider silk filled nerve conduits represent a favorable environment for Schwann cells and directional axon outgrowth.

5.3.2 Processed Silk Fibroin Fibers

Yang et al. introduced silk filaments in the lumen of a silk-based conduit that mimics an eggshell (Yang et al. 2007b). A fibrous silk mat served as inner wall for stiffness and a porous fibroin structure formed the outer wall. They implanted the graft in a 10 mm long rat sciatic nerve defect and demonstrated that axonal recovery and myelination approached the autologous nerve graft group after 6 months. A different silk-based conduit consisting of a highly porous fibroin scaffold filled with longitudinally orientated Spidrex[®] fibers was presented by Huang et al. (2012). The hyaluronic-acid-coated Spidrex[®] fibers served as excellent guiding structures for neurite outgrowth *in vitro* and were subsequently tested to bridge an 8 mm gap of a rat sciatic nerve. Of note, comparable axon outgrowth, myelination, target innervation, and functional recovery were observed between the autologous nerve graft and conduits containing 200 Spidrex[®] fibers up to 12 weeks post-surgery.

5.3.3 Silk-Based Hydrogels

Hydrogels are cross-linked polymer networks mimicking the fibrous as well as elastic structure of the extracellular environment. They are frequently used as soft substrates for (3D) cell culture and filling material for nerve conduits (Verdu et al. 2002; Sinis et al. 2005; Allmeling et al. 2008). Silk fibroin processing into hydrogels enables the design of three-dimensional porous structures with tunable stiffness that could provide a simple alternative to commercially available hydrogels. Hopkins et al. developed fibroin hydrogels that matched the nervous tissue rigidity. Compared to fibrin and collagen, the fibroin hydrogels provided superior structural integrity for DRG explant cultures (Hopkins et al. 2013). By controlling the self-assembly of silk

in aqueous solution, Bai et al. further tuned the rigidity of silk nanofibers to establish an optimal substrate for neuronal differentiation of nerve stem cells (Bai et al. 2014). Thus, silk-based hydrogels could replace commonly used ECM protein extracts such as Matrigel™, which is obtained from the murine Engelbreth–Holm–Swarm sarcoma and composed of a not well defined mixture of ECM proteins and growth factors that vary from batch to batch (Hughes et al. 2010). The combination of silk hydrogels with silk fibers as luminal filling for nerve conduits represents another promising approach for future studies.

5.3.4 Biofunctionalization of Silk-Based Conduits

Nerve regeneration depends on a complex interplay between cells, the ECM, and growth factors. While nerve conduits are constructed to establish an ECM-like scaffold, their modification with bioactive components aims to provide biological support for axonal regrowth. Numerous studies described beneficial regenerative effects of artificial nerve conduits enriched with cells, growth factors, ECM proteins, peptides, and electroconductive material, reviewed in (Magaz et al. 2018; Patel et al. 2018). Consequently, also silk-based scaffolds have been functionalized with biological components. A major advantage of aqueous fibroin solutions as primary source material for nerve conduit development is the easy biofunctionalization via different methods (Yoo et al. 2009). In the following, a brief overview about modified silk-based conduits is given.

5.3.5 Cellular Support

Supplementing artificial nerve conduits with cells such as Schwann cells or adipose-derived stem cells was shown to improve peripheral nerve regeneration presumably by providing contact guidance and growth factors (Hood et al. 2009; di Summa et al. 2010; Georgiou et al. 2015). The transplantation of autologous Schwann cells is considered the ideal cellular support as they are specialized to regulate the multistep process of nerve regeneration (Jessen and Mirsky 2016). Allmeling et al. supplemented a spider-silk-lined conduit with human Schwann cells dissolved in matrigel and demonstrated significantly increased regeneration when compared to cell-free controls 6 months after bridging a 20 mm sciatic nerve defect in rats (Allmeling et al. 2008). More recently, Das et al. pre-seeded Schwann cells on a silk-based gold nanocomposite conduit to bridge a 10 mm rat sciatic nerve defect (Das et al. 2015). The Schwann cell containing conduits improved structural and functional regeneration when compared to conduits without cellular support. However, the harvest of Schwann cells involves the sacrifice of healthy nerve tissue and donor site morbidity. Adipose-derived stem cells could offer a valid alternative because they are easily harvested, rapidly expanded, and secrete a variety of growth factors (Faroni et al. 2013). In addition, they can be differentiated into functional Schwann-like cells (Xie et al. 2017) and have been shown to improve peripheral nerve regeneration in a rat model (di Summa et al. 2010). Furthermore, Yang et al. investigated a silk-based nerve conduit containing silk filaments as luminal filler supplemented with bone marrow mesenchymal stem cells in a 10 mm long rat sciatic nerve defect (Yang et al. 2011). Functional and histological analysis after 12 weeks

showed a significant improvement of the regenerative outcome and increased Schwann cell content when compared to controls.

Although cell supplemented nerve conduits improved nerve regeneration in previous studies, cell-free conduits modified with growth factors, ECM proteins, peptide sequences, or other biological materials such as extracellular vesicles could avoid the disadvantages associated with autologous Schwann cell transplantations.

5.3.6 Growth Factors/Neurotrophic Factors

Schwann cells and the target tissues for neuronal innervation are the main sources of growth factors, by which they modulate nerve development, integrity, as well as regeneration. Three families of growth factors, i.e., neurotrophins, the CNTF family, and the GDNF family are referred to as neurotrophic factors that are able to specifically regulate the growth, survival, and differentiation of neuronal cells (Boyd and Gordon 2003). In line with their function, the incorporation of neurotrophic factors, alone or combined, into nerve conduit scaffolds was demonstrated to enhance sciatic nerve regeneration in rats; reviewed in (Pfister et al. 2007). Silk-based scaffolds containing neurotrophic factors can be obtained by blending the factors into a silk fibroin solution. Uebersax et al. generated a silk-fibroin-based film loaded with NGF and reported the successful release of bioactive NGF for more than 3 weeks (Uebersax et al. 2007). The NGF loaded silk films also supported neurite outgrowth during neuronal cell differentiation. Furthermore, fibroin hydrogels have been functionalized with the neurotrophic factor NT-3 (Hopkins et al. 2013). After 4 days of *in vitro* culture, the functionalized hydrogels increased neurite extension and axon bundling, and 11-day-old gels still released bioactive NT-3. In another work, electrospun fibroin mats loaded with GDNF and NGF showed sustained release of bioactive neurotrophic factors over 4 weeks (Madduri et al. 2010). Dinis et al. functionalized electrospun silk fibroin fibers with NGF and CNTF (Dinis et al. 2014). In relation to unfunctionalized fibers, primary rat DRG neurons responded to the NGF/CNTF fibers with a threefold increase in neurite length and increased Schwann cell alignment *in vitro*. Due to the lack of NGF release from the functionalized fibers, this study indicated that direct contact between the neurite and the NGF/CNTF incorporated fibers is responsible for neurotrophic factor uptake. Lin et al. evaluated the efficacy of a silk-fibroin-based nerve conduit containing silk microspheres loaded with GDNF in a 15 mm rat sciatic nerve defect (Lin et al. 2011). After 6 weeks, histological analysis demonstrated a significantly increased density of nerve tissue in animals with GDNF-containing microspheres compared to empty microspheres. Moreover, a concentration gradient of GDNF microspheres within the conduit increased Schwann cell migration as well as the density and number of axons. Hence, an improvement of nerve conduits can be achieved by implementing concentration gradients of neurotrophic factors (Magaz et al. 2018). However, it remains to be evaluated which manufacturing parameters, release mechanism, and combination of growth factors results in an ideal support of bioactive factors within artificial conduits for nerve repair.

5.3.7 ECM-Binding Proteins

Alternatively, nerve conduit scaffolds can be modified with laminin and fibronectin or other ECM proteins important for cellular activity (de Luca et al. 2014). Fibronectins connect the fibrous ECM scaffold with integrin receptors on the cell surface, which enables cell migration via cytoskeleton reorganization. Laminins are part of the basal lamina and assist in cell adhesion. Indeed, laminin is an essential growth substrate for Schwann cell cultures *in vitro* and stimulates their adhesion, survival, and proliferation (Vleggeert-Lankamp et al. 2004). DRGs cultured on fibronectin and laminin substrate also showed more rapid neurite elongation when compared to Poly-L-Lysine coating (Rogers et al. 1983). Functionalization of silk-based scaffolds can be undertaken by the covalent binding of laminin and fibronectin to silk fibroin. Mottaghitlab et al. modified a silk-based substrate with aligned fibronectin-containing fibroin nanofibers by electrospinning (Mottaghitlab et al. 2013). The produced conduit was used to bridge a 10 mm rat sciatic nerve defect. Compared to conduits without incorporated fibronectin, the immunohistochemistry evaluation showed an increased number of Schwann cells and myelinated axons 5 weeks after implantation. Recently, Schuh et al. functionalized silk fibroin with laminin and collagen isolated from human placental and detected significantly improved Schwann cell adhesion and proliferation in response to this substratum *in vitro* (Schuh et al. 2016).

5.3.8 Peptide Sequences

ECM proteins are commonly isolated from other organisms or tumor tissues, which limits their clinical use (Hersel et al. 2003). Thus, short peptide sequences mimicking the active binding domain of fibronectin and laminin have been generated and successfully shown to facilitate neurite outgrowth and selective neuronal differentiation *in vitro* (Schense et al. 2000; Hersel et al. 2003; Silva et al. 2004). In line with these findings, Sun et al. demonstrated enhanced neuronal differentiation and viability of neural stem cells in response to a silk-fibroin-based hydrogel modified with the Ile-Lys-Val-Ala-Val (IKVAV) motif found in laminin (Sun et al. 2017a). In addition, substrates containing the IKVAV cell binding motif fused to recombinant spider silk protein 4RepCT was shown to increase Schwann cell adherence and viability (Widhe et al. 2013). Silk fibroin could also be bound to the cell binding Arg-Gly-Asp (RGD) motif found in fibronectin for bone tissue engineering (Meinel et al. 2004). Taken together, cell binding peptide motifs represent promising adaptations for silk nerve conduits and await evaluation in animal studies.

6 Ongoing Preclinical Studies Using Silk-Based Nerve Conduits

There are a number of *in vivo* studies utilizing implantation of nerve conduits with linearly aligned silk fibers. From these studies, it has been well-established that nerve conduits with an inner material lining are superior to hollow tubes. In this regard, silk fibers can serve as an excellent material for nerve conduits to support guidance for outgrowing axons. Results indicate that implantation into peripheral nerves of these

nerve construct conduits can lead to enhanced axonal regeneration with improved functional recovery in a sciatic nerve model in rats. Two notable examples are from Rao and colleagues who described a hollow conduit with an outer sheath consisting of a hollow poly(lactic-co-glycolic acid) [PLGA] conduit filled with multiwalled silk fibroin/silk sericin as an internal skeleton (Rao et al. 2017), and Zhu et al. who developed a chitosan nerve guidance conduits (NGC) filled with silk fibroin filaments. Both of these models are very supportive of nerve regeneration (Zhu et al. 2018).

For repair of peripheral nerves in experimental *in vivo* settings, nerve conduits combined with silk fibroin have been used most frequently. Silk-based nerve conduits have favorable mechanical properties combined with tailorable degradation rates, thus providing a supportive microenvironment for nerve regeneration. The conduit itself in these silk-based systems consists of an outer sheath of combined silk or silk fibroin as scaffolding material or of a hollow tube with inner silk-based fibers as luminal fillers aligned in a linear array. Conduits made out of *Bombyx mori* silk protein filled with a silk-based biomaterial named Spidrex[®] with comparable characteristics of spider silk lead to functional recovery and axonal regeneration in a sciatic nerve model in rats. Noteworthy, high density of Spidrex[®] as the internal structure resulted in long distance nerve repair exceeding 10 mm (Huang et al. 2012).

A recent study in rats demonstrated that the combination of a poly (l-lactide-co-ε-caprolactone) [P(LLA-CL)] nerve conduit with silk fibroin is superior to a P(LLA-CL) conduit alone (Wang et al. 2018). The mechanism for this difference is unclear, but one possible effect responsible for the improved nerve regeneration in combination with silk fibroin might be an increase in angiogenesis which plays a crucial role in nerve repair especially within the nerve conduit (Wang et al. 2018). Moreover, silk-based fibroin conduits, which were optimized with appropriate porosity, demonstrated favorable immunogenicity with macrophage recruitment and subsequent release of interleukins resulting in increased axonal remyelination in a sciatic nerve defect model (Ghaznavi et al. 2011). Another approach by Sun et al. used Poly (l-lactic acid-co-ε-caprolactone)/silk fibroin nanofibers as nerve conduit fillers in a sciatic nerve model in rats (Sun et al. 2017b). In this study, the proliferation of Schwann cells, which infiltrated the conduit, was increased resulting in overall better nerve regeneration in comparison to control groups. A hybrid scaffold was developed by Gu and colleagues (Gu et al. 2014). Here, a chitosan conduit filled with silk fibroin (SF) fibers was prepared followed by Schwann cells (SC) seeding for extracellular matrix deposition (EDM). The conduit was then decellularized and implanted into a 10 mm sciatic nerve gap in rats. This chitosan/silk fibroin-based, by SC-derived ECM-modified, scaffold resulted in functionally and histologically superior regeneration compared to autografts. The engineering of polyaniline-silk fibroin (PASF) nanocomposite-based nerve conduits seeded with Schwann cells resulted in enhanced regeneration and functional outcome superior to nonseeded conduits in a 10 mm sciatic nerve model in rats (Das et al. 2017). To further optimize and accelerate nerve regeneration in long nerve defect injuries, silk-based conduits were enhanced with GDNF-loaded silk microspheres resulting *in vivo* in a higher density of regenerated nerve tissue and increased expression of the neuroendocrine marker PGP 9.5 protein in a supracritical nerve defect model in rats (Lin et al. 2011).

In addition to silk fibroin conduits, Teuschl et al. developed a nerve conduit based on processed and braided *Bombyx mori* silk filled with collagen to achieve a higher number of regenerated axons in a rat sciatic nerve injury model (Teuschl et al. 2015). To avoid immunological reaction to sericin, Allmeling et al. used unmodified, natural spider silk fibers longitudinally aligned in a decellularized vein as conduit resulting in highly effective nerve repair for bridging a long nerve gap in rabbits (Allmeling et al. 2008). All described studies above were performed in rodents or small animals with a nerve defect size between 10–15 mm. Two studies describing nerve regeneration after conduit implantation in a large animal model with larger nerve gap repair have been reported. In one, Xue et al. used an electrospun technique to develop a silk-fibroin-based conduit which was implanted in a 30 mm nerve gap in dog (Xue et al. 2018). Twelve months after implantation, satisfactory outcomes were observed. In another, a scaffold was inserted with silk fibers for bridging a nerve defect in sheep. Radtke et al. transplanted spider silk in decellularized veins in black head sheep as a preclinical model in a 6 cm critical size defect achieving results comparable to the gold standard of an autologous nerve graft thus demonstrating that spider silk is a good candidate for an alternative for autografting (Radtke et al. 2011).

7 Conclusions

Silk and silk fibroin possess favorable characteristics with regard to their flexibility, cell adhesion properties, and their superior biocompatibility and degradation properties compared to other materials. The possibility of integration of silk fibroin and silk into scaffolds, nanofibers, and membranes together with their characteristics make them excellent materials for tissue regeneration, repair, and tissue engineering. Moreover, with structural changes functionalization of the silk can be achieved to further enhance the tissue response leading to enhanced regenerative properties. Recent advancements are promising for further clinical translation and as a carrier material for bioactive drugs. These unique features have led to a variety of *in vitro* and *in vivo* experiments resulting in enhanced repair. Human trials need yet to be carried out, but existing experimental *in vivo* results are promising for future clinical applications. Silk and silk-based materials are an outstanding versatile substance and have shown promising results in a variety of tissue repair studies. The combination of silk-based conduits with cells or seeded pharmacological agents for peripheral nerve repair biomimicking nervous tissue provide further prospects for future tissue engineering and biomedical applications in clinical settings. In conclusion, silk with its unique properties is a promising material for translational research especially in challenging nerve gap repair.

References

- Agnarsson I, Kuntner M, Blackledge TA (2010) Bioprospecting finds the toughest biological material: extraordinary silk from a giant riverine orb spider. *PLoS One* 5(9):e11234
- Aigner TB, DeSimone E, Scheibel T (2018) Biomedical applications of recombinant silk-based materials. *Adv Mater* 30(19):e1704636

- Allmeling C, Jokuszies A, Reimers K, Kall S, Vogt PM (2006) Use of spider silk fibres as an innovative material in a biocompatible artificial nerve conduit. *J Cell Mol Med* 10(3):770–777
- Allmeling C, Jokuszies A, eimers K, Kall S, Choi CY, Brandes G, Kasper C, Scheper T, Guggenheim M, Vogt PM (2008) Spider silk fibres in artificial nerve constructs promote peripheral nerve regeneration. *Cell Prolif* 41(3):408–420
- Altman, G. H., F. Diaz, C. Jakuba, T. Calabro, R. L. Horan, . Chen, H. Lu, J. Richmond and D. L. Kaplan (2003). Silk-based biomaterials. *Biomaterials* 24(3): 401–416
- Arcidiacono S, Mello C, Kaplan D, Cheley S, Bayley H (1998) Purification and characterization of recombinant spider silk expressed in *Escherichia coli*. *Appl Microbiol Biotechnol* 49(1):31–38
- Arslantunali D, Dursun T, Yucel D, Hasirci N, Hasirci V (2014) Peripheral nerve conduits: technology update. *Med Devices (Auckl)* 7:405–424
- Arthur-Farraj PJ, Latouche M, Wilton DK, Quintes S, Chabrol E, Banerjee A, Woodhoo A, Jenkins B, Rhman M, Turmaine M, Wicher GK, Mitter R, Greensmith L, Behrens A, Raivich G, Mirsky R, Jessen KR (2012) C-Jun reprograms Schwann cells of injured nerves to generate a repair cell essential for regeneration. *Neuron* 75(4):633–647
- Bai S, Zhang W, Lu Q, Ma Q, Kaplan DL, Zhu H (2014) Silk nanofiber hydrogels with tunable modulus to regulate nerve stem cell fate. *J Mater Chem B* 2(38):6590–6600
- Barr LA, Fahnestock SR, Yang J (2004) Production and purification of recombinant DP1B silk-like protein in plants. *Mol Breed* 13(4):345–356
- Bini E, Foo CW, Huang J, Karageorgiou V, Kitchel B, Kaplan DL (2006) RGD-functionalized bioengineered spider dragline silk biomaterial. *Biomacromolecules* 7(11):3139–3145
- Blamires SJ, Blackledge TA, Tso IM (2017) Physicochemical property variation in spider silk: ecology, evolution, and synthetic production. *Annu Rev Entomol* 62:443–460
- Bogush VG, Sokolova OS, Davydova LI, Klinov DV, Sidoruk KV, Esipova NG, Neretina TV, Orchanskyi IA, Makeev VY, Tumanyan VG, Shaitan KV, Debabov VG, Kirpichnikov MP (2009) A novel model system for design of biomaterials based on recombinant analogs of spider silk proteins. *J Neuroimmune Pharmacol* 4(1):17–27
- Boyd JG, Gordon T (2003) Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. *Mol Neurobiol* 27(3):277–324
- Brooks AE, Nelson SR, Jones JA, Koenig C, Hinman M, Stricker S, Lewis RV (2008) Distinct contributions of model MaSp1 and MaSp2 like peptides to the mechanical properties of synthetic major ampullate silk fibers as revealed in silico. *Nanotechnol Sci Appl* 1:9–16
- Brown CN, Finch JG (2010) Which mesh for hernia repair? *Ann R Coll Surg Engl* 92(4):272–278
- Cameron HD (2005) Chapter 73: an etymological dictionary of North American spider genus names. In: *Spiders of North America: an identification manual*. American Arachnological Society, New Hampshire
- Cao Y, Wang B (2009) Biodegradation of silk biomaterials. *Int J Mol Sci* 10(4):1514–1524
- Cao TT, Zhang YQ (2016) Processing and characterization of silk sericin from *Bombyx mori* and its application in biomaterials and biomedicines. *Mater Sci Eng C Mater Biol Appl* 61:940–952
- Chen P, Tong XL, Fu MY, Hu H, Song JB, He SZ, Gai TT, Dai FY, Lu C (2016) Molecular mapping and characterization of the silkworm apodal mutant. *Sci Rep* 6:18956
- Conrad U, Fiedler U (1998) Compartment-specific accumulation of recombinant immunoglobulins in plant cells: an essential tool for antibody production and immunomodulation of physiological functions and pathogen activity. Springer, Dordrecht
- Craig CL (1997) Evolution of arthropod silks. *Annu Rev Entomol* 42:231–267
- Craig CL, Hsu M, Kaplan D, Pierce NE (1999) A comparison of the composition of silk proteins produced by spiders and insects. *Int J Biol Macromol* 24(2–3):109–118
- Crapo PM, Gilbert TW, Badylak SF (2011) An overview of tissue and whole organ decellularization processes. *Biomaterials* 32(12):3233–3243
- Cregg J (2007) DNA-mediated transformation. *Methods Mol Biol* 389:27–42
- Cunniff PM, Fossey SA, Auerbach MA, Song JW, Kaplan DL, Adams WW, Eby RK, Mahoney D, Vezie DL (1994) Mechanical and thermal properties of dragline silk from the spider *Nephila clavipes*. *Polym Adv Technol* 5(8):401–410
- Daroff RBJ, Joseph, Mazziotta JC, Pomeroy SL, Bradley WG (2016) *Bradley's neurology in clinical practice*. Elsevier, New York

- Das S, Sharma M, Saharia D, Sarma KK, Sarma MG, Borthakur BB, Bora U (2015) In vivo studies of silk based gold nano-composite conduits for functional peripheral nerve regeneration. *Bio-materials* 62:66–75
- Das S, Sharma M, Saharia D, Sarma KK, Muir EM, Bora U (2017) Electrospun silk-polyaniline conduits for functional nerve regeneration in rat sciatic nerve injury model. *Biomed Mater* 12 (4):045025
- de Luca AC, Lacour SP, Raffoul W, di Summa PG (2014) Extracellular matrix components in peripheral nerve repair: how to affect neural cellular response and nerve regeneration? *Neural Regen Res* 9(22): 1943–1948
- DeFrancesco L (2017) Hanging on a thread. *Nat Biotechnol* 35:496–499
- di Summa PG, Kingham PJ, Raffoul W, Wiberg M, Terenghi G, Kalbermatten DF (2010) Adipose-derived stem cells enhance peripheral nerve regeneration. *J Plast Reconstr Aesthet Surg* 63 (9):1544–1552
- Disin TM, Vidal G, Jose RR, Vigneron P, Bresson D, Fitzpatrick V, Marin F, Kaplan DL, Egles C (2014) Complementary effects of two growth factors in multifunctionalized silk nanofibers for nerve reconstruction. *PLoS One* 9(10):e109770
- Disin TM, Elia R, Vidal G, Dermigny Q, Denoed C, Kaplan DL, Egles C, Marin F (2015) 3D m channel bi-functionalized silk electrospun conduits for peripheral nerve regeneration. *J Mech Behav Biomed Mater* 41:43–55
- El-Bassyouni H, Mohammed M. Ahmed (2018). Genome editing: a review of literature, LAP LAMBERT Academic Publishing, Beau Bassin, Mauritius
- Elbert DL (2011) Bottom-up tissue engineering. *Curr Opin Biotechnol* 22(5):674–680
- Evans GR, Brandt K, Katz S, Chauvin P, Otto L, Bogle M, Wang B, Meszlenyi RK, Lu L, Mikos AG, Patrick CW Jr (2002) Bioactive poly(L-lactic acid) conduits seeded with Schwann cells for peripheral nerve regeneration. *Biomaterials* 23(3):841–848
- Faroni A, Terenghi G, Reid AJ (2013) Adipose-derived stem cells and nerve regeneration: promises and pitfalls. *Int Rev Neurobiol* 108:121–136
- Foelix RF (1996) *Biology of spiders*. Oxford University Press, New York
- Freddi G, Mossotti R, Innocenti R (2003) Degumming of silk fabric with several proteases. *J Biotechnol* 106(1):101–112
- Gage LP, Manning RF (1980) Internal structure of the silk fibroin gene of *Bombyx mori*. I the fibroin gene consists of a homogeneous alternating array of repetitious crystalline and amorphous coding sequences. *J Biol Chem* 255(19):9444–9450
- Gatesy J, Hayashi C, Motriuk D, Woods J, Lewis R (2001) Extreme diversity, conservation, and convergence of spider silk fibroin sequences. *Science* 291(5513):2603–2605
- Gellynck K, Verdonk P, Forsyth R, Almqvist K, Van Nimmen E, Gheysens T, Mertens J, Van Langenhove L, Kiekens P, Verbruggen G (2008) Biocompatibility and biodegradability of spider egg sac silk. *J Mater Sci Mater Med* 19(8):2963–2970
- Georgiou M, Golding JP, Loughlin AJ, Kingham PJ, Phillips JB (2015) Engineered neural tissue with aligned, differentiated adipose-derived stem cells promotes peripheral nerve regeneration across a critical sized defect in rat sciatic nerve. *Biomaterials* 37:242–251
- Gerritsen VB (2002) The tiptoe of an airbus. *Prot Spot* 24:1–2
- Ghaznavi AM, Kokai LE, Lovett ML, Kaplan DL, Marra KG (2011) Silk fibroin conduits: a cellular and functional assessment of peripheral nerve repair. *Ann Plast Surg* 66(3):273–279
- Goldman IL, Kadulin SG, Razin SV (2004) Transgenic animals in medicine: integration and expression of foreign genes, theoretical and applied aspects. *Med Sci Monit* 10(11):Ra274–Ra285
- Goldsmith MR, Shimada T, Abe H (2005) The genetics and genomics of the silkworm, *Bombyx mori*. *Annu Rev Entomol* 50(1):71–100
- Gosline JM, Guerette PA, Ortlepp CS, Savage KN (1999) The mechanical design of spider silks: from fibroin sequence to mechanical function. *J Exp Biol* 202(23):3295–3303
- Grinsell D, Keating CP (2014) Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. *Biomed Res Int* 2014:698256

- Gu Y, Zhu J, Xue C, Li Z, Ding F, Yang Y, Gu X (2014) Chitosan/silk fibroin-based, Schwann cell-derived extracellular matrix-modified scaffolds for bridging rat sciatic nerve gaps. *Biomaterials* 35(7):2253–2263
- Gyori E, Radtke C, Gordon T, Borschel GH (2018) [Pathway protection – enhanced motoneuron regeneration by end-to-side coaptation of sensory axons]. *Handchir Mikrochir Plast Chir* 50 (5):341–347
- Haastert K, Joswig H, Jaschke KA, Samii M, Grothe C (2010) Nerve repair by end-to-side nerve coaptation: histologic and morphometric evaluation of axonal origin in a rat sciatic nerve model. *Neurosurgery* 66(3):567–576. discussion 576–567
- Hayashi CY, Lewis RV (2000) Molecular architecture and evolution of a modular spider silk protein gene. *Science* 287(5457):1477–1479
- Heidebrecht A, Scheibel T (2013) Chapter four – recombinant production of spider silk proteins. *Adv Appl Microbiol* 82:115–153
- Heim M, Keerl D, Scheibel T (2009) Spider silk: from soluble protein to extraordinary fiber. *Angew Chem Int Ed Eng* 48(20):3584–3596
- Hersel U, Dahmen C, Kessler H (2003) RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. *Biomaterials* 24(24):4385–4415
- Hinman MB, Lewis RV (1992) Isolation of a clone encoding a second dragline silk fibroin. *Nephila clavipes* dragline silk is a two-protein fiber. *J Biol Chem* 267(27):19320–19324
- Hoke A (2006) Mechanisms of disease: what factors limit the success of peripheral nerve regeneration in humans? *Nat Clin Pract Neurol* 2(8):448–454
- Hood B, Levene HB, Levi AD (2009) Transplantation of autologous Schwann cells for the repair of segmental peripheral nerve defects. *Neurosurg Focus* 26(2):E4
- Hopkins AM, De Laporte L, Tortelli F, Spedden E, Staii C, Atherton TJ, Hubbell JA, Kaplan DL (2013) Silk hydrogels as soft substrates for neural tissue engineering. *Adv Funct Mater* 23 (41):5140–5149
- Horan RL, Antle K, Collette AL, Wang Y, Huang J, Moreau JE, Volloch V, Kaplan DL, Altman GH (2005) In vitro degradation of silk fibroin. *Biomaterials* 26(17):3385–3393
- Hu A, Zuo B, Zhang F, Lan Q, Zhang H (2012) Electrospun silk fibroin nanofibers promote Schwann cell adhesion, growth and proliferation. *Neural Regen Res* 7(15):1171–1178
- Hu A, Zuo B, Zhang F, Zhang H, Lan Q (2013) Evaluation of electrospun silk fibroin-based transplants used for facial nerve repair. *Otol Neurotol* 34(2):311–318
- Huang W, Begum R, Barber T, Ibba V, Tee NC, Hussain M, Arastoo M, Yang Q, Robson LG, Lesage S, Gheysens T, Skaer NJ, Knight DP, Priestley JV (2012) Regenerative potential of silk conduits in repair of peripheral nerve injury in adult rats. *Biomaterials* 33(1):59–71
- Hughes CS, Postovit LM, Lajoie GA (2010) Matrigel: a complex protein mixture required for optimal growth of cell culture. *Proteomics* 10(9):1886–1890
- Hundepool CA, Nijhuis TH, Kotsougiani D, Friedrich PF, Bishop AT, Shin AY (2017) Optimizing decellularization techniques to create a new nerve allograft: an in vitro study using rodent nerve segments. *Neurosurg Focus* 42(3):E4
- Inoue S-i, Tsuda H, Tanaka T, Kobayashi M, Yoshiko M, Magoshi J (2003) Nanostructure of natural fibrous protein: in vitro nanofabric formation of *Samia cynthia ricini* wild silk fibroin by self-assembling. *Nano Lett* 3(10):1329–1332
- Iridag Y, Kazanci M (2006) Preparation and characterization of *Bombyx mori* silk fibroin and wool keratin. *J Appl Polym Sci* 100(5):4260–4264
- Jena K, Pandey JP, Kumari R, Sinha AK, Gupta VP, Singh GP (2018) Tasar silk fiber waste sericin: new source for anti-elastase, anti-tyrosinase and anti-oxidant compounds. *Int J Biol Macromol* 114:1102–1108
- Jessen KR, Mirsky R (2016) The repair Schwann cell and its function in regenerating nerves. *J Physiol* 594(13):3521–3531
- Jessen KR, Mirsky R, Arthur-Farraj P (2015) The role of cell plasticity in tissue repair: adaptive cellular reprogramming. *Dev Cell* 34(6):613–620

- Jiang X, Lim SH, Mao HQ, Chew SY (2010) Current applications and future perspectives of artificial nerve conduits. *Exp Neurol* 223(1):86–101
- Johnson EO, Zoubos AB, Soucacos PN (2005) Regeneration and repair of peripheral nerves. *Injury* 36(Suppl 4):S24–S29
- Kanno H, Pearce DD, Ozawa H, Itoi E, Bunge MB (2015) Schwann cell transplantation for spinal cord injury repair: its significant therapeutic potential and prospectus. *Rev Neurosci* 26(2):121–128
- Knight DP, Vollrath F (2001) Changes in element composition along the spinning duct in a *Nephila* spider. *Naturwissenschaften* 88(4):179–182
- Kornfeld T, Vogt PM, Bucan V, Peck CT, Reimers K, Radtke C (2016) Characterization and schwann cell seeding of up to 15.0 cm long spider silk nerve conduits for reconstruction of peripheral nerve defects. *J Funct Biomater* 7(4):30
- Kovoor J (1987) Comparative structure and histochemistry of silk-producing organs in arachnids. In: Nentwig W (ed) *Ecophysiology of spiders*. Springer, Berlin, Heidelberg, pp 160–186
- Kuhbier JW, Reimers K, Kasper C, Allmeling C, Hillmer A, Menger B, Vogt PM, Radtke C (2011) First investigation of spider silk as a braided microsurgical suture. *J Biomed Mater Res B Appl Biomater* 97B(2):381–387
- Kukuruzinska MA, Lennon K (1998) Protein N-glycosylation: molecular genetics and functional significance. *Crit Rev Oral Biol Med* 9(4):415–448
- Kundu B, Rajkhowa R, Kundu SC, Wang X (2013) Silk fibroin biomaterials for tissue regenerations. *Adv Drug Deliv Rev* 65(4):457–470
- Kurioka A, Yamazaki M (2002) Antioxidant in the Cocoon of the silkworm, *Bombyx mori*. *J Insect Biotechnol Sericology* 71(3):177–180
- Lally KP, Cheu HW, Vazquez WD (1993) Prosthetic diaphragm reconstruction in the growing animal. *J Pediatr Surg* 28(1):45–47
- Lefevre T, Boudreault S, Cloutier C, Pezolet M (2011) Diversity of molecular transformations involved in the formation of spider silks. *J Mol Biol* 405(1):238–253
- Lin YC, Ramadan M, Hronik-Tupaj M, Kaplan DL, Philips BJ, Sivak W, Rubin JP, Marra KG (2011) Spatially controlled delivery of neurotrophic factors in silk fibroin-based nerve conduits for peripheral nerve repair. *Ann Plast Surg* 67(2):147–155
- Liu Y, Sponner A, Porter D, Vollrath F (2008) Proline and processing of spider silks. *Biomacromolecules* 9(1):116–121
- Lloyd AW (2002) Interfacial bioengineering to enhance surface biocompatibility. *Med Device Technol* 13(1):18–21
- Lovett ML, Cannizzaro CM, Vunjak-Novakovic G, Kaplan DL (2008) Gel spinning of silk tubes for tissue engineering. *Biomaterials* 29(35):4650–4657
- Madduri S, Papaloizos M, Gander B (2010) Trophically and topographically functionalized silk fibroin nerve conduits for guided peripheral nerve regeneration. *Biomaterials* 31(8):2323–2334
- Madsen B, Shao ZZ, Vollrath F (1999) Variability in the mechanical properties of spider silks on three levels: interspecific, intraspecific and intraindividual. *Int J Biol Macromol* 24(2–3):301–306
- Magaz A, Faroni A, Gough JE, Reid AJ, Li X, Blaker JJ (2018) Bioactive silk-based nerve guidance conduits for augmenting peripheral nerve repair. *Adv Healthc Mater* 7(23):e1800308
- Maksimenco OG, Deykin AV, Khodarovich YM, Georgiev PG (2013) Use of transgenic animals in biotechnology: prospects and problems. *Acta Nat* 5(1):33–46
- Mandal BB, Kundu SC (2008) A novel method for dissolution and stabilization of non-mulberry silk gland protein fibroin using anionic surfactant sodium dodecyl sulfate. *Biotechnol Bioeng* 99(6):1482–1489
- Mason C, Dunnill P (2008) A brief definition of regenerative medicine. *Regen Med* 3(1):1–5
- Matsumoto K, Uejima H, Iwasaki T, Sano Y, Sumino H (1996) Studies on regenerated protein fibers. III. Production of regenerated silk fibroin fiber by the self-dialyzing wet spinning method. *J Appl Polym Sci* 60(4):503–511

- Meinel L, Karageorgiou V, Hofmann S, Fajardo R, Snyder B, Li C, Zichner L, Langer R, Vunjak-Novakovic G, Kaplan DL (2004) Engineering bone-like tissue in vitro using human bone marrow stem cells and silk scaffolds. *J Biomed Mater Res A* 71(1):25–34
- Melke J, Midha S, Ghosh S, Ito K, Hofmann S (2016) Silk fibroin as biomaterial for bone tissue engineering. *Acta Biomater* 31:1–16
- Millesi H, Schmidhammer R (2007) End-to-side coaptation – controversial research issue or important tool in human patients. *Acta Neurochir Suppl* 100:103–106
- Min BM, Lee G, Kim SH, Nam YS, Lee TS, Park WH (2004) Electrospinning of silk fibroin nanofibers and its effect on the adhesion and spreading of normal human keratinocytes and fibroblasts in vitro. *Biomaterials* 25(7–8):1289–1297
- Mita K, Ichimura S, James TC (1994) Highly repetitive structure and its organization of the silk fibroin gene. *J Mol Evol* 38(6):583–592
- Moloney MM, Holbrook LA (1997) Subcellular targeting and purification of recombinant proteins in plant production systems. *Biotechnol Genet Eng Rev* 14:321–336
- Mondal M, Trivedy K, Kumar N (2006) The silk proteins, sericin and fibroin in silkworm, *Bombyx mori* Linn. – a review. *J Env Sci* 5(2):63–76
- Moore AM, Kasukurthi R, Magill CK, Farhadi HF, Borschel GH, Mackinnon SE (2009) Limitations of conduits in peripheral nerve repairs. *Hand (N Y)* 4(2):180–186
- Moore AM, MacEwan M, Santosa KB, Chenard KE, Ray WZ, Hunter DA, Mackinnon SE, Johnson PJ (2011) Acellular nerve allografts in peripheral nerve regeneration: a comparative study. *Muscle Nerve* 44(2):221–234
- Mottaghitalab F, Farokhi M, Zaminy A, Kokabi M, Soleimani M, Mirahmadi F, Shokrgozar MA, Sadeghizadeh M (2013) A biosynthetic nerve guide conduit based on silk/SWNT/fibronectin nanocomposite for peripheral nerve regeneration. *PLoS One* 8(9):e74417
- Muheremu A, Ao Q (2015) Past, present, and future of nerve conduits in the treatment of peripheral nerve injury. *Biomed Res Int* 2015:237507
- Nazarov R, Jin HJ, Kaplan DL (2004) Porous 3-D scaffolds from regenerated silk fibroin. *Biomacromolecules* 5(3):718–726
- Newman J, Newman C (1995) Oh what a tangled web: the medicinal uses of spider silk. *Int J Dermatol* 34(4):290–292
- Nichol JW, Khademhosseini A (2009) Modular tissue engineering: engineering biological tissues from the bottom up. *Soft Matter* 5(7):1312–1319
- Ovid, Humphries R (1983) *Metamorphoses*. Indiana University Press, Bloomington
- Padamwar M, Pawar A (2004) Silk sericin and its applications: a review. *J Sci Ind Res* 63(4):323–329
- Patel NP, Lyon KA, Huang JH (2018) An update-tissue engineered nerve grafts for the repair of peripheral nerve injuries. *Neural Regen Res* 13(5):764–774
- Pérez-Rigueiro J, Elices M, Llorca J, Viney C (2001) Tensile properties of silkworm silk obtained by forced silking. *J Appl Polym Sci* 82(8):1928–1935
- Pérez-Rigueiro J, Elices M, Llorca J, Viney C (2002) Effect of degumming on the tensile properties of silkworm (*Bombyx mori*) silk fiber. *J Appl Polym Sci* 84(7):1431–1437
- Pérez-Rigueiro J, Elices M, Plaza G, Real JI, Guinea GV (2005) The effect of spinning forces on spider silk properties. *J Exp Biol* 208(14):2633–2639
- Pfister LA, Papaliozos M, Merkle HP, Gander B (2007) Nerve conduits and growth factor delivery in peripheral nerve repair. *J Peripher Nerv Syst* 12(2):65–82
- Putthananat S, Stribeck N, Fossey SA, Eby RK, Adams WW (2000) Investigation of the nanofibrils of silk fibers. *Polymer* 41(21):7735–7747
- Qi Y, Wang H, Wei K, Yang Y, Zheng R-Y, Kim IS, Zhang K-Q (2017) A review of structure construction of silk fibroin biomaterials from single structures to multi-level structures. *Int J Mol Sci* 18(3):237
- Qu J, Wang D, Wang H, Dong Y, Zhang F, Zuo B, Zhang H (2013) Electrospun silk fibroin nanofibers in different diameters support neurite outgrowth and promote astrocyte migration. *J Biomed Mater Res A* 101(9):2667–2678

- Radtke C (2016) Natural occurring silks and their analogues as materials for nerve conduits. *Int J Mol Sci* 17(10):1754
- Radtke C, Allmeling C, Waldmann K-H, Reimers K, Thies K, Schenk HC, Hillmer A, Guggenheim M, Brandes G, Vogt PM (2011) Spider silk constructs enhance axonal regeneration and remyelination in long nerve defects in sheep. *PLoS One* 6(2):e16990
- Ramshaw JAM, Werkmeister JA, Dumsday GJ (2014) Bioengineered collagens. *Bioengineered* 5(4):227–233
- Rao J, Cheng Y, Liu Y, Ye Z, Zhan B, Quan D, Xu Y (2017) A multi-walled silk fibroin/silk sericin nerve conduit coated with poly(lactic-co-glycolic acid) sheath for peripheral nerve regeneration. *Mater Sci Eng C Mater Biol Appl* 73:319–332
- Ray WZ, Mackinnon SE (2010) Management of nerve gaps: autografts, allografts, nerve transfers, and end-to-side neurorrhaphy. *Exp Neurol* 223(1):77–85
- Rhisiart A a, Vollrath F (1994) Design features of the orb web of the spider, *Araneus diadematus*. *Behav Ecol* 5(3):280–287
- Rising A, Widhe M, Johansson J, Hedhammar M (2011) Spider silk proteins: recent advances in recombinant production, structure-function relationships and biomedical applications. *Cell Mol Life Sci* 68(2):169–184
- Rockwood DN, Preda RC, Yucel T, Wang X, Lovett ML, Kaplan DL (2011) Materials fabrication from *Bombyx mori* silk fibroin. *Nat Protoc* 6(10):1612–1631
- Rogers SL, Letourneau PC, Palm SL, McCarthy J, Furcht LT (1983) Neurite extension by peripheral and central nervous system neurons in response to substratum-bound fibronectin and laminin. *Dev Biol* 98(1):212–220
- Roloff F, Strauss S, Vogt PM, Bicker G, Radtke C (2014) Spider silk as guiding biomaterial for human model neurons. *Biomed Res Int* 2014:906819
- Rosenberg AH, Goldman E, Dunn JJ, Studier FW, Zubay G (1993) Effects of consecutive AGG codons on translation in *Escherichia coli*, demonstrated with a versatile codon test system. *J Bacteriol* 175(3):716–722
- Schafer-Nolte F, Hennecke K, Reimers K, Schnabel R, Allmeling C, Vogt PM, Kuehner JW, Mirastschijski U (2014) Biomechanics and biocompatibility of woven spider silk meshes during remodeling in a rodent fascia replacement model. *Ann Surg* 259(4):781–792
- Scheibel T (2004) Spider silks: recombinant synthesis, assembly, spinning, and engineering of synthetic proteins. *Microb Cell Factories* 3(1):14
- Schense JC, Bloch J, Aebischer P, Hubbell JA (2000) Enzymatic incorporation of bioactive peptides into fibrin matrices enhances neurite extension. *Nat Biotechnol* 18(4):415–419
- Schlosshauer B, Dreesmann L, Schaller HE, Sinis N (2006) Synthetic nerve guide implants in humans: a comprehensive survey. *Neurosurgery* 59(4):740–747. discussion 747–748
- Schuh CM, Monforte X, Hackethal J, Redl H, Teuschl AH (2016) Covalent binding of placental derived proteins to silk fibroin improves schwann cell adhesion and proliferation. *J Mater Sci Mater Med* 27(12):188
- Seal BL, Otero TC, Panitch A (2001) Polymeric biomaterials for tissue and organ regeneration. *Mater Sci Eng R Rep* 34(4):147–230
- Sehnal F, Akai H (1990) Insect silk glands: their types, development and function, and effects of environmental factors and morphogenetic hormones on them. *Int J Insect Morphol Embryol* 19(2):79–132
- Silva GA, Czeisler C, Niece KL, Beniash E, Harrington DA, Kessler JA, Stupp SI (2004) Selective differentiation of neural progenitor cells by high-epitope density nanofibers. *Science* 303(5662):1352–1355
- Sinis N, Schaller HE, Schulte-Eversum C, Schlosshauer B, Doser M, Dietz K, Rosner H, Muller HW, Haerle M (2005) Nerve regeneration across a 2-cm gap in the rat median nerve using a resorbable nerve conduit filled with Schwann cells. *J Neurosurg* 103(6):1067–1076
- Soong HK, Kenyon KR (1984) Adverse reactions to virgin silk sutures in cataract surgery. *Ophthalmology* 91(5):479–483

- Sorensen HP, Mortensen KK (2005) Advanced genetic strategies for recombinant protein expression in *Escherichia coli*. *J Biotechnol* 115(2):113–128
- Soumya M, Harinatha Reddy A, Nageswari G, Venkatappa B (2017) Silkworm (*Bombyx mori*) and its constituents: A fascinating insect in science and research. *J Entomol Zool Stud* 5(5):1701–1705
- Spohner A, Schlott B, Vollrath F, Unger E, Grosse F, Weisshart K (2005) Characterization of the protein components of *Nephila clavipes* dragline silk. *Biochemistry* 44(12):4727–4736
- Sulaiman OA, Gordon T (2009) Role of chronic Schwann cell denervation in poor functional recovery after nerve injuries and experimental strategies to combat it. *Neurosurgery* 65(4 Suppl):A105–A114
- Sun W, Yu H, Shen Y, Banno Y, Xiang Z, Zhang Z (2012) Phylogeny and evolutionary history of the silkworm. *Sci China Life Sci* 55(6):483–496
- Sun W, Incitti T, Migliaresi C, Quattrone A, Casarosa S, Motta A (2017a) Viability and neuronal differentiation of neural stem cells encapsulated in silk fibroin hydrogel functionalized with an IKVAV peptide. *J Tissue Eng Regen Med* 11(5):1532–1541
- Sun B, Zhou Z, Wu T, Chen W, Li D, Zheng H, El-Hamshary H, Al-Deyab SS, Mo X, Yu Y (2017b) Development of nanofiber sponges-containing nerve guidance conduit for peripheral nerve regeneration in vivo. *ACS Appl Mater Interfaces* 9(32):26684–26696
- Sutherland TD, Young JH, Weisman S, Hayashi CY, Merritt DJ (2010) Insect silk: one name, many materials. *Annu Rev Entomol* 55(1):171–188
- Tang X, Ding F, Yang Y, Hu N, Wu H, Gu X (2009) Evaluation on in vitro biocompatibility of silk fibroin-based biomaterials with primarily cultured hippocampal neurons. *J Biomed Mater Res A* 91(1):166–174
- Teh TKH, Toh S-L, Goh JCH (2010) Optimization of the silk scaffold sericin removal process for retention of silk fibroin protein structure and mechanical properties. *Biomed Mater* 5(3):035008
- Teuschl AH, Schuh C, Halbweis R, Pajer K, Marton G, Hopf R, Mosia S, Runzler D, Redl H, Nogradi A, Hausner T (2015) A new preparation method for anisotropic silk fibroin nerve guidance conduits and its evaluation in vitro and in a rat sciatic nerve defect model. *Tissue Eng Part C Methods* 21(9):945–957
- Thiel BL, Guess KB, Viney C (1997) Non-periodic lattice crystals in the hierarchical microstructure of spider (major ampullate) silk. *Biopolymers* 41(7):703–719
- Tokareva O, Jacobsen M, Buehler M, Wong J, Kaplan DL (2014) Structure-function-property-design interplay in biopolymers: spider silk. *Acta Biomater* 10(4):1612–1626
- Uebachs L, Mattotti M, Papalozos M, Merkle HP, Gander B, Meinel L (2007) Silk fibroin matrices for the controlled release of nerve growth factor (NGF). *Biomaterials* 28(30):4449–4460
- Unger RE, Wolf M, Peters K, Motta A, Migliaresi C, James Kirkpatrick C (2004) Growth of human cells on a non-woven silk fibroin net: a potential for use in tissue engineering. *Biomaterials* 25(6):1069–1075
- Vepari C, Kaplan DL (2007) Silk as a biomaterial. *Prog Polym Sci* 32(8–9):991–1007
- Verdu E, Labrador RO, Rodriguez FJ, Ceballos D, Fores J, Navarro X (2002) Alignment of collagen and laminin-containing gels improve nerve regeneration within silicone tubes. *Restor Neurol Neurosci* 20(5):169–179
- Vleggeert-Lankamp CL, Pego AP, Lakke EA, Deenen M, Marani E, Thomeer RT (2004) Adhesion and proliferation of human Schwann cells on adhesive coatings. *Biomaterials* 25(14):2741–2751
- Vollrath F (1992) Spider webs and silks. *Sci Am* 266(3):70–77
- Vollrath F (1993) General properties of some spider silks. In: *Silk polymers*, American chemical society symposium series 544, Washington, DC, pp 17–28
- Vollrath F (2000) Strength and structure of spiders' silks. *J Biotechnol* 74(2):67–83
- Vollrath F (2005) Spiders' webs. *Curr Biol* 15(10):R364–R365
- Vollrath F, Porter D (2006) Spider silk as archetypal protein elastomer. *Soft Matter* 2(5):377–385
- Wang HB, Mullins ME, Cregg JM, McCarthy CW, Gilbert RJ (2010) Varying the diameter of aligned electrospun fibers alters neurite outgrowth and Schwann cell migration. *Acta Biomater* 6(8):2970–2978

- Wang C, Jia Y, Yang W, Zhang C, Zhang K, Chai Y (2018) Silk fibroin enhances peripheral nerve regeneration by improving vascularization within nerve conduits. *J Biomed Mater Res A* 106(7):2070–2077
- Wen H, Lan X, Zhang Y, Zhao T, Wang Y, Kajiura Z, Nakagaki M (2010) Transgenic silkworms (*Bombyx mori*) produce recombinant spider dragline silk in cocoons. *Mol Biol Rep* 37(4):1815–1821
- Whitlock EL, Tuffaha SH, Luciano JP, Yan Y, Hunter DA, Magill CK, Moore AM, Tong AY, Mackinnon SE, Borschel GH (2009) Processed allografts and type I collagen conduits for repair of peripheral nerve gaps. *Muscle Nerve* 39(6):787–799
- Widhe M, Johansson U, Hillerdahl CO, Hedhammar M (2013) Recombinant spider silk with cell binding motifs for specific adherence of cells. *Biomaterials* 34(33):8223–8234
- Work RW (1976) The force-elongation behavior of web fibers and silks forcibly obtained from orb-web-spinning spiders. *Text Res J* 46(7):485–492
- Wu HC, Quan DN, Tsao CY, Liu Y, Terrell JL, Luo X, Yang JC, Payne GF, Bentley WE (2017) Conferring biological activity to native spider silk: a biofunctionalized protein-based microfiber. *Biotechnol Bioeng* 114(1):83–95
- Xia X-X, Qian Z-G, Ki CS, Park YH, Kaplan DL, Lee SY (2010) Native-sized recombinant spider silk protein produced in metabolically engineered *Escherichia coli* results in a strong fiber. *Proc Natl Acad Sci* 107(32):14059–14063
- Xia Q, Li S, Feng Q (2014) Advances in silkworm studies accelerated by the genome sequencing of *Bombyx mori*. *Annu Rev Entomol* 59(1):513–536
- Xie F, Zhang H, Shao H, Hu X (2006) Effect of shearing on formation of silk fibers from regenerated *Bombyx mori* silk fibroin aqueous solution. *Int J Biol Macromol* 38(3–5):284–288
- Xie S, Lu F, Han J, Tao K, Wang H, Simental A, Hu D, Yang H (2017) Efficient generation of functional Schwann cells from adipose-derived stem cells in defined conditions. *Cell Cycle* 16(9):841–851
- Xu H-T, Fan B-L, Yu S-Y, Huang Y-H, Zhao Z-H, Lian Z-X, Dai Y-P, Wang L-L, Liu Z-L, Fei J, Li N (2007) Construct synthetic gene encoding artificial spider dragline silk protein and its expression in Milk of transgenic mice. *Anim Biotechnol* 18(1):1–12
- Xu J, Dong Q, Yu Y, Niu B, Ji D, Li M, Huang Y, Chen X, Tan A (2018) Mass spider silk production through targeted gene replacement in *Bombyx mori*. *Proc Natl Acad Sci* 115(35):8757–8762
- Xue C, Zhu H, Tan D, Ren H, Gu X, Zhao Y, Zhang P, Sun Z, Yang Y, Gu J, Gu Y, Gu X (2018) Electrospun silk fibroin-based neural scaffold for bridging a long sciatic nerve gap in dogs. *J Tissue Eng Regen Med* 12(2):e1143–e1153
- Yang Y, Shao Z, Chen X, Zhou P (2004) Optical spectroscopy to investigate the structure of regenerated *Bombyx mori* silk fibroin in solution. *Biomacromolecules* 5(3):773–779
- Yang Y, Chen X, Ding F, Zhang P, Liu J, Gu X (2007a) Biocompatibility evaluation of silk fibroin with peripheral nerve tissues and cells in vitro. *Biomaterials* 28(9):1643–1652
- Yang Y, Ding F, Wu J, Hu W, Liu W, Liu J, Gu X (2007b) Development and evaluation of silk fibroin-based nerve grafts used for peripheral nerve regeneration. *Biomaterials* 28(36):5526–5535
- Yang Y, Yuan X, Ding F, Yao D, Gu Y, Liu J, Gu X (2011) Repair of rat sciatic nerve gap by a silk fibroin-based scaffold added with bone marrow mesenchymal stem cells. *Tissue Eng Part A* 17(17–18):2231–2244
- Yip EC, Rayor LS (2014) Maternal care and subsocial behaviour in spiders. *Biol Rev* 89(2):427–449
- Yoo HS, Kim TG, Park TG (2009) Surface-functionalized electrospun nanofibers for tissue engineering and drug delivery. *Adv Drug Deliv Rev* 61(12):1033–1042
- Zarkoob S, Eby RK, Reneker DH, Hudson SD, Adams WW (2004) Structure and morphology of electrospun silk nanofibers. *Polymer* 45(11):3973–3977
- Zeplin PH, Maksimovikj NC, Jordan MC, Nickel J, Lang G, Leimer AH, Römer L, Scheibel T (2014) Spider silk coatings as a bioshield to reduce Periprosthetic fibrous capsule formation. *Adv Funct Mater* 24(18):2658–2666

- Zhang Y-Q (2002) Applications of natural silk protein sericin in biomaterials. *Biotechnol Adv* 20 (2):91–100
- Zhang X, Reagan MR, Kaplan DL (2009) Electrospun silk biomaterial scaffolds for regenerative medicine. *Adv Drug Deliv Rev* 61(12):988–1006
- Zhang Q, Zhao Y, Yan S, Yang Y, Zhao H, Li M, Lu S, Kaplan DL (2012) Preparation of uniaxial multichannel silk fibroin scaffolds for guiding primary neurons. *Acta Biomater* 8(7):2628–2638
- Zhu C, Huang J, Xue C, Wang Y, Wang S, Bao S, Chen R, Li Y, Gu Y (2018) Skin derived precursor Schwann cell-generated acellular matrix modified chitosan/silk scaffolds for bridging rat sciatic nerve gap. *Neurosci Res* 135:21–31
- Zuo B, Dai L, Wu Z (2006) Analysis of structure and properties of biodegradable regenerated silk fibroin fibers. *J Mater Sci* 41(11):3357–3361

Collagen Biomaterials for Nerve Tissue Engineering

Despoina Eleftheriadou and James B. Phillips

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Abstract

Proteins in the collagen family represent some of the most versatile natural biomaterials used in neural tissue engineering. Their widespread use is mainly prompted by their favorable physicochemical properties, rich biological activity, and abundant expression in the native extracellular matrix. This chapter presents an overview of the various applications of collagen-based biomaterials developed for neural tissue engineering.

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1 Introduction

1.1 Collagen in Native Tissues: Function and Diversity

Collagen is the most abundant protein in mammals (Fratzl 2008; Shoulders and Raines 2009; Frantz et al. 2010). As a natural component of the extracellular matrix (ECM), it is involved in a number of essential biological activities such as network formation, scaffolding, morphogenesis, and injury repair (Barros et al. 2011; Ricard-Blum 2011). In addition, collagen has been one of the most evolutionarily conserved proteins in vertebrates (Stover and Verrelli 2010). Up to date, 29 different collagen molecules have been identified in the interstitial and pericellular matrices or as transmembranous proteins (see Table 1) (Gordon and Hahn 2010; Lin et al. 2019). The various collagen subtypes are characterized by a notable complexity and heterogeneity in their structure, their splice variants, and the presence of different domains. They also present differences in their self-organization process, and as a result, display different functions (Fig. 1).

Fibril-forming collagens, including types I, II, and III, are the most widespread among the different subtypes (Karsdal 2019), being expressed in most connective tissues in the human body. As structural subunits, fibrillar collagens have an integral role in preserving the mechanical integrity and three-dimensional form of different tissues and organs and offer a functional extracellular environment for cell growth (Mouw et al. 2014; Bella and Hulmes 2017). Nonfibrillar collagen proteins can be further separated into various subcategories, such as network-forming (collagen types IV and VII), multiple triple-helix domains and interruptions (MULTI-PLEXINs), fibril-associated collagens with interrupted triple helices (FACITs, including types IX and XII), and membrane-associated collagens with interrupted triple helices (MACITs) (Kadler et al. 2007; Shoulders and Raines 2009; Theocharis et al. 2016). These all have diverse roles. For instance, collagenous transmembrane proteins, including collagen XVII, XIII, and XXV, are an emerging group of versatile biomolecules with dual functionality as both cell surface receptors and cell signaling molecules (Franzke et al. 2003).

1.2 Structure and Biosynthesis

All subtypes of collagen, whether fibrillar or not, possess a highly organized and hierarchical structure and are formed by the same basic units. The secondary structure of collagen is comprised of amino acid chains which can either be identical or a combination of two or more individual α chains creating a heterotrimer; heterotypic triple helices are more prevalent than homotrimeric ones. Each α -chain is characterized by the presence of at least one collagenous domain, containing the sequence (Gly-X-Y), where X and Y could be substituted by any amino acid (Hulmes 2008). Glycine is the smallest amino acid and is positioned at the center

Table 1 Collagen types, modified from (Gelse et al. 2003; Karsdal 2019; Lin et al. 2019)

Type	Molecular form	Tissue localization	Primary function (s)
<i>I</i>	Fibrillar	Noncartilaginous connective tissues including bone, cornea, dermis, dentine, ligaments, nerve, sclera, tendons, and blood vessels	Structural protein: maintains tissue architecture and integrity
<i>II</i>	Fibrillar	Articular and hyaline cartilage, vitreous body, and nuclei pulposi	Structural protein: maintains tissue architecture and integrity
<i>III</i>	Fibrillar	Skin, vessel walls, reticular fibers of most tissues (lung, liver, spleen, intestines, etc.) Often associated with type I collagen, with the exception of bone	Structural protein: maintains tissue architecture and integrity
<i>IV</i>	Network-forming	Basement membranes including the glomeruli of the kidney, ear cochlea, lung, skin, and bronchial epithelium	Separation of different tissue compartments Angiogenesis Presynaptic organizer
<i>V</i>	Fibrillar	Ubiquitous including fetal membranes, bone, lungs, skeletal muscle, and cornea	Structural protein: maintains tissue architecture and integrity
<i>VI</i>	Beaded filaments	Muscle, placenta, lung, intervertebral disc, vessel wall, dermis, brain, and cartilage	Structural component Associated with protection of neurons against amyloid-beta toxicity
<i>VII</i>	Anchoring fibrils	Skin, colon, dermal-epidermal junctions, placenta, oral mucosa, cornea, and cervix	Preserving dermal-epidermal adhesion
<i>VIII</i>	Hexagonal network forming	Endothelial cells, Descemet's membrane, cartilage, brain, and heart	Structural and signaling component
<i>IX</i>	FACIT	Hyaline cartilage, vitreous humor, intervertebral discs, and cornea; same as type II	Maintaining tissue integrity
<i>X</i>	Hexagonal network forming	Hypertrophic cartilage and growth plate	Regulating endochondral ossification
<i>XI</i>	Fibrillar	Same as type II	Structural component
<i>XII</i>	FACIT	Perichondrium, ligaments, skin tendon, and periodontal ligament	Not clear
<i>XIII</i>	Transmembrane	Epidermis, hair follicle, endomysium, intestine, chondrocyte, lung, liver, dermo-epidermal junction, and neuromuscular structures	Regulation of osteogenesis
<i>XIV</i>	FACIT	Skin, tendon, vessel wall, placenta, lungs, liver, and articular cartilage	Maintaining mechanical strength

(continued)

Table 1 (continued)

Type	Molecular form	Tissue localization	Primary function (s)
<i>XV</i>	MULTIPLEXIN	Fibroblast, smooth muscle cell, kidney, and pancreas	Not clear
<i>XVI</i>	FACIT	Fibroblast, amnion, and keratinocyte	Structural component
<i>XVII</i>	Transmembrane	Dermal-epidermal junction	Structural component
<i>XVIII</i>	MULTIPLEXIN	Lung, liver, vascular, and epithelial basement membrane	Associated with eye development and basement membrane integrity
<i>XIX</i>	FACIT	Breast, colon, central neurons, spleen, kidney, and human rhabdomyosarcoma	Associated with hippocampal synapses Smooth muscle dysfunction
<i>XX</i>	FACIT	Corneal epithelium, embryonic skin, sternum cartilage, and tendon	Not clear
<i>XXI</i>	FACIT	Heart, placenta, stomach, jejunum, skeletal muscle, kidney, lung, pancreas, and lymph node	Not clear
<i>XXII</i>	FACIT	Myotendinous junctions especially in heart and skeletal muscle, cartilage-synovial fluid, and hair follicle-dermis	Stabilization of tissue junctions
<i>XXIII</i>	Transmembrane	Prostate, lung, skin, tendon, and amnion	Linked to prostate cancer
<i>XXIV</i>	Fibrillar	Predominantly in bone, less in cornea, ovary, testis, and liver	Regulating osteoblast differentiation and bone formation
<i>XXV</i>	Transmembrane	Precursor protein for collagenous Alzheimer amyloid plaque component Low-level expression in the heart, testis, and eye	Associated with Alzheimer's disease
<i>XXVI</i>	Beaded filaments	Testis and ovary	Developmental processes
<i>XXVII</i>	Fibrillar	Mostly in cartilage, also in skin, cornea, retina, and major arteries of the heart	Cartilage calcification
<i>XXVIII</i>	Beaded filaments	Peripheral nerves and skin calvaria	Not clear
<i>XXIX</i>	Beaded filaments	Skin, lung, small intestine, and colon	Associated with atopic dermatitis

of the protein, thereby allowing the close packing of the three chains. The X-Y residues are exposed on the surface of the protein and enable the formation of interchain hydrogen bonds. To enhance the stability of tertiary structures, a significant fraction of the X and Y positions should be occupied by amino acids with secondary amino groups, most frequently by prolines and hydroxyprolines (Persikov

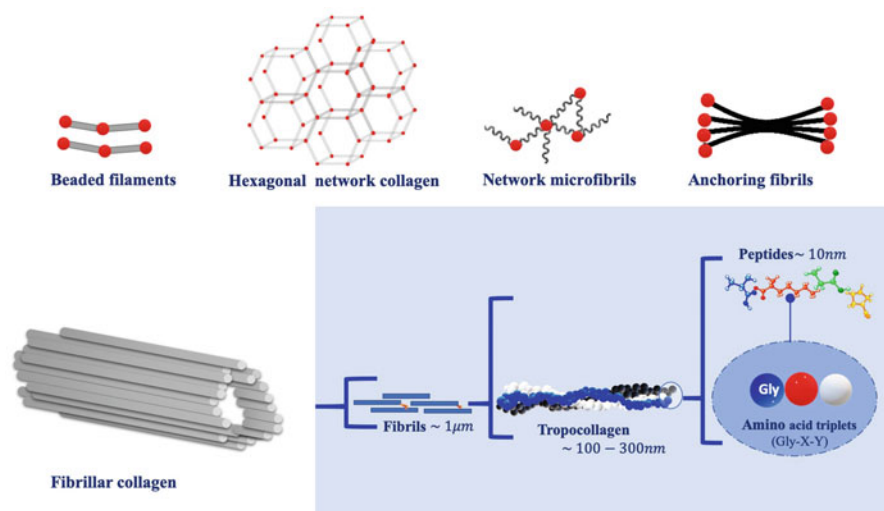


Fig. 1 Schematic representation of different types of collagen molecules. The insert depicts the hierarchical features of fibrillar collagen

et al. 2000). Based on the type of collagen, the triple helices can be a major or minor component of the molecule (Beck and Brodsky 1998; Ramshaw et al. 1998; Hulmes 2008). The rest of the regions may contain different noncollagenous domains. Although the presence of a triple-helix is a prerequisite feature for a protein to be classified as collagen, it is not a sufficient one (Ricard-Blum 2011). To avoid confusion, the collagen family is restricted to triple helical proteins forming a right-handed complex and having distinct roles in tissue formation, organization, or maintenance.

Another defining feature of a large part of the collagen protein family is their ability to self-assemble into fibers. Supramolecular assembly of fibrillar collagen is governed by the formation of intermolecular cross-links that ultimately bestow upon collagen fibers remarkable elasticity and resistance to mechanical forces (Gutsmann et al. 2004). The self-assembly mechanism is regulated *in vivo* by enzymes and chemical interactions between propeptides on protein molecules (Myllyharju and Kivirikko 2004; Robins 2007). During *in vivo* genesis of fibrillar collagen, the three parallel helical α -chains self-assemble around each other and generate a right-handed triple helix procollagen molecule (Ramachandran and Kartha 1954; Cowan et al. 1955; Rich and Crick 1955). At this point, the procollagens go through several modifications (mainly hydroxylation and glycosylation) before finally being assembled into triple helical structures (Ishikawa and Bächinger 2013). Procollagen peptidase then removes loose termini to create tropocollagen molecules. These subsequently undergo an entropy-driven fibrillogenesis process and covalently bind to each other to form microfibrils (Prockop and Fertala 1998). Finally, electrostatic and hydrophobic forces drive the attraction between collagen microfibrils which stack together to form larger fibrils (Zhu et al. 2018). As an end result,

collagen fibers with diameter up to several microns are developed (Kadler et al. 2007; Orgel et al. 2011). Hydrogen bonding between collagen molecules is facilitated by water that is an essential component in the collagen self-assembly mechanism and helps maintain the molecular conformation (Suzuki et al. 1980).

1.3 Collagen as a Biomaterial

Due to its vital role in tissue, collagen has long been utilized as a natural polymer for biomedical applications, either as native, unmodified grafts or as advanced medical products. Applications of collagen date back to the Roman Empire, where Galen of Pergamon first proposed its use as sutures to repair wounds and injured tendons in gladiators (Snyder 1976; Nutton 2012). Nevertheless, the rise in the popularity of collagen-based biomaterials was a product of twentieth century technological advancements and scientific investigations that allowed us to understand its chemistry and biology. For instance, it was the insight in purification and characterization methods (Nageotte 1927; Cassel and Kanagy 1949) that established core technologies for collagen extraction and production of related products with reproducible properties. Then, in the early 1930s and 1950s, the interaction between cells and hydrogels fabricated from extracted collagen was first studied (Huzella 1932; Ehrmann and Gey 1956), which paved the way for more advanced products. To date, collagen-based biomaterials have been introduced in wound care (Chattopadhyay and Raines 2014; Davison-Kotler et al. 2019), orthopedic and sports medicine (Cunniffe and O'Brien 2011), dentistry (Patino et al. 2002), aesthetic medicine and reconstructive surgery (van Wachem et al. 2001; Cockerham and Hsu 2009), ophthalmology (Gouveia and Connon 2016), cardiovascular medicine (Copes et al. 2019), and as drug delivery system (Wallace and Rosenblatt 2003; Zhang et al. 2018).

The widespread utilization of collagen in biomedical applications is underpinned by several favorable properties: low immunogenicity and toxicity, cell-binding domains and signaling elements, hydrophilic nature, robust biocompatibility, and biodegradability (Parenteau-Bareil et al. 2010). From a structural viewpoint, the extensive hydrogen bonds within collagen contribute to its high tensile strength, and the X and Y residues on the surface make functionalization with other biomolecules relatively easy. Furthermore, the ease of isolation and purification of collagen along with the fact that it is highly conserved between species makes it a readily available biomaterial.

Commonly used xenogeneic collagen sources include porcine or equine tissues (Gorlov et al. 2018; Terzi et al. 2019), calfskin and tendons (Rodrigues et al. 2003), kangaroos (Johnson et al. 1999), rat tail tendons (Rajan et al. 2006), and alligator bones (Wood et al. 2008). Collagen can also be isolated from various marine life forms such as jellyfish (Song et al. 2006), making it a relatively straightforward and cost-effective solution for tissue engineering. Nevertheless, there are still issues with the quality, heterogeneity, potential batch-to-batch variability (Browne et al. 2013), and the risk of transmission of infectious agents (Asher 1999; Patino et al. 2003) of

animal-derived collagen. Therefore, efforts have been directed toward obtaining sufficient amounts of different types of highly purified human recombinant collagen (Bulleid et al. 2000; Yang et al. 2004). A number of transgenic organisms and bioreactor-based eukaryotic systems, such as yeast (Toman et al. 2000; Olsen et al. 2001), insects (Myllyharju et al. 1997), tobacco plants (Stein et al. 2009), and *Escherichia Coli* (Rutschmann et al. 2014) have been successfully used for the expression of triple-helical human collagen resistant to thermal denaturation. Despite their enhanced performance and superior safety, the low yields and prohibitive prices, instability, and lack of customizability of recombinant collagens limit their use. Until these problems are resolved, researchers are unlikely to routinely adopt recombinant human collagen for tissue engineering (Browne et al. 2013).

1.4 Collagen Fabrication and Functionalization for Tissue Engineering Applications

Generally, for engineered tissues fabricated from natural biomaterials to exhibit optimal clinical functionality, it is desirable that critical native biomimetic features remain intact (Chen and Liu 2016). Therefore, finding ways to preserve the biological and physicochemical properties of collagen *ex vivo* is particularly important for tissue engineering applications (Lin et al. 2019). Given the vast variety of collagen-based biomaterials and their synthesis methods, discussing all of them is beyond the scope of this chapter. Instead, this section will focus on two main categories that are particularly relevant to nerve tissue engineering, decellularized extracellular matrix (dECM)-based scaffolds, and reconstituted collagenous biomaterials. The following sections will briefly summarize the principles behind them. More detailed information regarding the processing and development of advanced collagen biomaterials can be found in the following reviews (Zhu et al. 2018; Meyer 2019; Lin et al. 2019).

1.4.1 Decellularized Collagen Biomaterials

Decellularization tends to yield dECM in which the predominant component is collagen, with architecture, collagen type, and presence of other components varying according to the source material and the decellularization process. *In vivo*, the recruitment and infiltration of native cells into engineered constructs is greatly affected by their physicochemical properties such as porosity, mesh network architecture, anisotropy, and stiffness (Nelson and Bissell 2006; Leipzig and Shoichet 2009; Zhu et al. 2019). In this regard, scaffolds derived from dECM exhibit notable advantages over those made from synthetic materials. Besides their beneficial architectural and biochemical complexity, the degradation products of dECM scaffolds have been shown to promote neoangiogenesis, trigger a favorable immune reaction by encouraging macrophages to acquire a proregenerative macrophage phenotype (M2), and induce a positive tissue remodeling response (Meng et al. 2015; Tukmachev et al. 2016; Ren et al. 2018). Therefore, over the past decades, mammalian tissue and whole organ decellularization have gained considerable attention. A relevant example of dECM used in nerve tissue regeneration is the

off-the-shelf allogeneic decellularized nerve graft, Avance[®] from AxoGen[®]. In this case, donor human nerve tissue is harvested, and its cellular content is removed, while retaining the native microstructure and cell-guiding potential (Hudson et al. 2004). These processed allografts are inherently nonimmunogenic and retain some of the internal laminin structure and neurotrophic factors of native nerve tissue (Whitlock et al. 2009).

Decellularization techniques are broadly categorized into enzymatic, physical, and chemical methods, with a combination of two or more being used if necessary (Gilbert 2012). Despite their differences, all of them aim to remove cells, related components, and host antigens, without disrupting the microstructure and biological activity of the ECM. Maintaining the mechanical integrity of the ECM and avoiding removal of bioactive factors are particularly important for cellular growth, differentiation, and repair (Aamodt and Grainger 2016). The selection of an optimal decellularization protocol to produce clinically relevant scaffolds depends on the intended purpose. For instance, while ionic detergents such as sodium dodecyl sulphate (SDS) have been shown consistently to remove over 90% of genetic material (Pang et al. 2010; Zhou et al. 2010; Wang et al. 2010; Jank et al. 2015), they can render the resulting materials cytotoxic (Fernández-Pérez and Ahearne 2019). The use of SDS can also irreversibly damage the ultrastructure of the collagen network and lead to significantly reduced elastic modulus and tensile strength (Xu et al. 2014; Hwang et al. 2017; Liu et al. 2018). With regard to enzymatic decellularization, collagen proteins are particularly sensitive to the enzymes used, and thus, such processes should be performed with appropriate care (Kasimir et al. 2003; Brown et al. 2010). Given the issues with the techniques mentioned above, physical decellularization using mechanical dissociation, supercritical carbon dioxide treatment, or freeze-thawing cycles might be more desirable (Karabekmez et al. 2009; Crapo et al. 2012; Guler et al. 2017; Seo et al. 2018).

1.4.2 Reconstituted Collagen Biomaterials

Engineered neural tissue-like substitutes composed of natural polymer hydrogels have been shown to support nerve regeneration both *in vitro* and *in vivo* (Georgiou et al. 2013; Quigley et al. 2013; Shahriari et al. 2016; Meyer et al. 2016). Hence, several approaches have focused on manipulating the mechanisms driving fibrillogenesis to develop three-dimensional hydrogel-based materials (Zhu et al. 2018). The traditional method to prepare such hydrogels relies on the ability of collagens to change their behavior and self-assembly depending on the pH, ionic concentration, temperature, and other environmental conditions to which they are exposed. For instance, it has been observed that collagen starts to disassemble into monomers at lower pH levels, with the disaggregation process being reversible. Therefore, collagen hydrogels can be fabricated by neutralizing a weakly acidic aqueous solution of purified collagen and warming it to approximately 30 °C. This rapid and easy two-step synthesis protocol harnesses the intrinsic ability of collagen monomers to self-assemble under physiological conditions (Cooper 1970; Li et al. 2009; Harris et al. 2013).

To better recapitulate the mechanical and structural properties of the native neural tissue, collagen hydrogels used for peripheral nerve conduits are often blended with other polymers, stabilized by plastic compression or cross-linked by irradiation or enzymes (Cheema and Brown 2013; Davidenko et al. 2015; Schuh et al. 2018). Moreover, specialized molds with micropatterns, sacrificial templating, and bioprinting are often employed to produce collagen hydrogels with more defined morphologies that closely mimic those of the native microenvironment (Yanagawa et al. 2016; Li and Gao 2018; Jiang et al. 2020).

Fiber alignment and anisotropy can also be achieved by the application of an external magnetic field (Guo and Kaufman 2007) or electrical current (Cheng et al. 2008), shear flow deposition in a microfluidic chamber (Lee et al. 2006), or simply by placing the collagen solution in a unidirectional rocker during setting (Fauzi et al. 2016). However, most of these methods are elaborate and require expert knowledge and highly specialized and costly equipment, which makes them difficult to scale up. Mechanical forces can also be used to generate aligned collagen hydrogels. Alignment toward a specific orientation can be induced by the application of anisotropic stress, which can be introduced to the scaffolds either by an externally applied force or via cell-mediated contraction (Roeder et al. 2002; Girtton et al. 2002; Vader et al. 2009; Georgiou et al. 2013; Kubow et al. 2015).

When fibrous constructs with varying degrees of porosity, precise internal architecture, and alignment are desired, alternative fabrication methods such as freeze-drying, or electrospinning, can be employed. Freeze-drying is a well-established method for synthesizing collagen-based scaffolds with controllable pore architecture. The process works by freezing an aqueous suspension of collagen to create a network with interpenetrating ice crystals. Then, the environmental pressure surrounding the scaffold is decreased to encourage the ice formed to sublime, thereby yielding a structure with interconnected pores (Haugh et al. 2009; Lowe et al. 2016). Pore size and orientation can be controlled by using temperature gradients or preformed ice micropattern templates (Davidenko et al. 2012; Oh et al. 2012). The main disadvantage of freeze-drying is that it is time-consuming and requires considerable energy supply which significantly increases its cost. Electrospinning is another promising technique that can be used to generate highly aligned collagen fiber structures. Electrospinning is performed by applying a voltage to a polymer or melt solution. When the applied electrostatic force is sufficient to overcome the surface tension of the solution, it draws charged threads of the protein and produces fibers with diameters ranging from microns to nanometers (Castilla-Casadio et al. 2017). It should be noted though, that the volatile solvents, high shear, and high voltages often used during electrospinning could irreversibly damage collagen's periodic structure, resulting in denatured gelatin fibers (Zeugolis et al. 2008; Dong et al. 2009). Constructs with architecture reminiscent of the native nerve tissue can also be produced by wet-spinning (Siriwardane et al. 2014) and solvent or thermally induced phase separation (Liu et al. 2015). Finally, macroscopic fibrillar scaffolds can be produced by extrusion (Pins and Silver 1995), a method first described by Kato et al. in 1989 (Kato et al. 1989). This is a simple, effortless, and easily scalable way to produce tubular collagen scaffolds with patterned fiber alignment and

geometry. Yet, it is often overlooked by the scientific community. The process parameters, such as flow rate, collagen concentration, vessel diameter, and needle gauge, can be further optimized to obtain scaffolds with the desired morphological characteristics. Recently, Kamranpour et al. designed a gel aspiration-ejection (GAE) system that could produce stable collagen scaffolds with controlled micro-structure, alignment, density, and mechanical properties (Kamranpour et al. 2016).

2 Collagen in Nerve Tissue Engineering

2.1 Collagen in Healthy and Diseased Peripheral Nerves

So far, two main classes of collagen proteins have been identified in the peripheral nervous system (PNS), those expressed in the layers of connective tissue that surround the neural cells (mostly types I, III, and V), and those located within basement membranes (mostly type IV) (Koopmans et al. 2009). These are present at varying levels within the perineurium, epineurium, and endoneurium. In addition, collagens have a prominent role in PNS development and regeneration as well as in the maintenance of normal function of mature peripheral nerves (Hubert et al. 2009). Some distinctive functions of the different collagen types in the PNS are shown in Table 2.

During neural development, collagens are involved in axonal guidance and synaptogenesis. For instance, collagens I, IV, IX, and XVIII are considered favorable substrates for supporting the growth of extending neurites and directing them to their targets (Ring et al. 1996; Schneider and Granato 2006; Xiao and Baier 2007; Leclere et al. 2007). Collagens also provide a structural framework for peripheral nerves. Both the endoneurium and the perineurium contain type I and III collagen, with the respective fibrils being morphologically and biochemically different (Osawa and Ide 1986; Ushiki and Ide 1990). Variation in the diameter of collagen fibrils in different locations within nerves has been linked to localized stiffness changes associated with anatomical features such as joints (Mason and Phillips 2011). Particularly, crucial roles have also been suggested for collagen VI and to a lesser extent collagens IV and V in regulating Schwann cell migration, differentiation, and function, and thus, subsequent myelination (Chen et al. 2015; Gregorio et al. 2018). Finally, collagen XXVIII, which is unusually confined in the PNS (Veit et al. 2006), has been found to surround all nonmyelinating glial cells of the somatosensory system. In the case of myelinated nerve fibers, its expression is highly localized in the nodes of Ranvier and terminal branches (Grimal et al. 2010). Its role in the PNS remains elusive.

It was established quite early that differential collagen expression is implicated in regeneration following a peripheral nerve injury (PNI) (Seyer et al. 1977; Siironen et al. 1992). Certain collagen types, such as collagen type I and III produced by fibroblasts, are believed to promote neurite outgrowth by providing mechanical support. Others are implicated in the terminal differentiation of Schwann cells and the regulation of myelination. Yet, excessive collagen formation and/or improper

Table 2 Location and function of various types of collagen proteins in peripheral nerves. (Modified from Hubert et al. 2009; Chen et al. 2015)

Collagen type	Location	Function
<i>Collagens I and III</i>	Epineurium, perineurium, and endoneurium	Structural support and neurite outgrowth
<i>Collagen II</i>	Expressed by both myelinating and nonmyelinating Schwann cells	Unclear
<i>Collagen IV</i>	Basement membrane	Associated with Schwann cells and vasculature. Promotes cell adhesion, migration, and proliferation, as well as axonal growth Also present at neuromuscular junction development
<i>Collagen V</i>	Expressed by Schwann cells	Inhibits axonal outgrowth. Essential for myelination
<i>Collagen VI</i>	Associated with Schwann cells, macrophages, and endoneurial/perineurial cells	Involved in Schwann cell differentiation and myelination
<i>Collagen IX</i>	Expressed by the sclerotome cells of the somite	PNS segmentation Restricts the outgrowth of sensory and motor neurons, and neural crest cell migration
<i>Collagen XIII and XIV</i>	Dorsal root ganglia and nerves in development (based on in vivo studies)	Unclear
<i>Collagen XV</i>	Endoneurium, perineurium, and Schwann cell basement membrane of neuromuscular junction	Associated with PNS maturation, nerve fiber organization, and sensory function of peripheral nerves
<i>Collagen XVI</i>	Dorsal root ganglia	Unclear
<i>Collagen XXVIII</i>	Nodes of Ranvier and nonmyelinating cells	Unclear

reorganization after neurotrauma can act as a mechanical barrier to nerve regeneration (Zochodne 2000; Koopmans et al. 2009).

2.2 Collagen-Based Nerve Guidance Conduits: Preclinical Studies

The use of nerve guidance constructs (NGCs) was originally proposed as a possible nerve repair strategy in 1880, with the first *in vivo* study occurring in 1882. The first *in vivo*-implanted NGC was a hollow conduit derived from decalcified bone used to bridge a 30 mm nerve gap (reviewed by Ijpma et al. 2008)). Today, artificial nerve tissue substitutes have the potential to become an affordable and versatile alternative to the “gold standard” autograft nerve repair (di Summa et al. 2014).

Material selection is a key parameter of NGC design. To ensure a successful repair, NGCs should be nontoxic, have an appropriate resorption rate, and be flexible

and minimally immunogenic (Stang et al. 2009). NGCs should also a) be stable enough to provide support and prevent nerve compression, b) limit scar formation, c) allow unrestricted diffusion of nutrients, and d) encourage axon growth, Schwann cell migration, and revascularization (Bozkurt et al. 2012; Pixley et al. 2016). Mechanical properties of the selected biomaterial also need to be considered prior to NGC fabrication and implantation, as this could be critical for determining their regenerative potential (Bartlett et al. 2020). First, at cellular level, mechanical properties may influence the behavior and phenotype of exogenous and endogenous cells. Substrate stiffness is a crucial parameter that affects the attachment, proliferation, and lineage commitment of implanted therapeutic cells (Georges et al. 2006; Engler et al. 2006; Leipzig and Shoichet 2009). It has also been found to affect the behavior of Schwann cells and the extension of neurites, which are highly mechanosensitive (Hoffman-Kim et al. 2010; Rosso et al. 2017). Mechanical properties of a biomaterial can also determine the host tissue response, by inducing the recruitment and activation of macrophages and other antigen presenting cells (Kim et al. 2014; Friedemann et al. 2017). Second, at the macroscopic level, forces occurring at the biomaterial-tissue interface could disrupt structural continuity and prevent axons from crossing. From a practical perspective, the implanted NGCs must have adequate strength and compliance to ensure proper load transfer and allow surgical handling and suturing. The selected biomaterial must be able to endure and resist the tensile, shear, and compressive stresses that native nerves can accommodate.

Even though in practice there is no single biomaterial that satisfies all the requirements, collagen represents a promising candidate, mainly due to its inherent biocompatibility and low antigenicity. The potential of two- and three-dimensional collagen substrates to encourage neurogenesis and axonal elongation and the relationship between growth rate and substrate stiffness was demonstrated decades ago (Hirose et al. 1993; Krewson et al. 1994; Willits and Skornia 2004). Type I and III collagen matrices were found to be particularly effective in supporting the outgrowth of chick sympathetic ganglia (Carri et al. 1992).

2.2.1 First-Generation Collagen NGCs: Hollow Conduits

The first collagen-based NGCs consisted of hollow tubes, had a relatively simple design, and were characterized by adequate mechanical strength and permeability. When implanted in rodents (4 or 6 mm nerve gaps) and nonhuman primates (5 or 15 mm nerve gaps), they supported axonal regeneration and led to functional outcomes close or equivalent to autografts after 3 months and 800 days respectively (Archibald et al. 1991, 1995; Gómez et al. 1996). These early *in vivo* studies are the basis for the development of the first commercially available collagen-based nerve-repair device, NeuraGen™. NeuraGen™ is manufactured by Integra Lifesciences (Princeton, NJ) and was approved by the FDA in 2001. Despite the popularity of hollow collagen NGCs, results regarding their clinical efficacy have been inconsistent and in the case of large nerve gaps relatively poor (Thomsen et al. 2010; Wangenstein and Kalliainen 2010).

2.2.2 Second-Generation Collagen NGCs

Intraluminal Fillers

Emerging biological knowledge regarding axonal regeneration highlighted the need for more complex NGC designs. Therefore, the incorporation of various topographic, biological, physical, and chemotactic cues to guide neurite extension and create a growth permissive microenvironment was examined (Dewle et al. 2020). One of the early approaches employed cross-linked highly porous tubes made of collagen-glycosaminoglycan copolymers encapsulated in silicone tubes. These were shown to support regeneration across 15 mm rat sciatic nerve gaps (Yannas et al. 1985). Subsequently, several types of lumen fillers were developed and tested, including hydrogels based on other ECM components (Valentini et al. 1987), therapeutic cells (Ansselin et al. 1997), and resorbable substrates (Itoh et al. 2001).

Anisotropy and Guidance Structures

Early observations demonstrated that magnetically aligned collagen fibers incorporated into bioresorbable collagen NGCs could enhance regeneration of rat sciatic nerves (Ceballos et al. 1999), providing impetus for the incorporation of organized structural elements into the lumen of NGCs. For instance, Keilhoff et al. developed NGCs from porcine collagen I and III with encapsulated Schwann cells or inner collagen skeletons and implanted them into rat sciatic nerve lesions. These were well-tolerated and successfully integrated into the host tissue. Angiogenesis was observed between 5 and 7 days after implantation. This rapid vascularization was proposed to contribute to the survival of co-transplanted Schwann cells that proliferated on the inner surface of the conduits and formed nerve-guiding cellular columns (Keilhoff et al. 2003). Despite their biocompatibility, implantation of these conduits in 20 mm rat sciatic nerve gaps resulted in poor regeneration in comparison to the autograft (Stang et al. 2005).

As the field progressed, relevant research yielded more advanced methods to introduce alignment and anisotropy into NGCs. Verdú et al. subjected collagen hydrogels to gravitational or magnetic forces to induce longitudinal alignment of their fibrils. The resulting hydrogels were then inserted into silicone tubes and implanted into a 6 mm mouse sciatic nerve gap. Results indicated a correlation between fibril alignment and improved recovery and reinnervation (Verdú et al. 2002). In an alternative approach, the self-alignment of Schwann cells within tethered collagen hydrogels provided a means to generate anisotropic cellular hydrogels within silicone tubes, which improved regeneration in a 5 mm rat sciatic nerve gap compared with empty silicone tubes (Phillips et al. 2005). Based on this concept, Georgiou et al. developed engineered neural tissue (EngNT), a cellular type I collagen hydrogel tethered to induce cell-mediated contraction and alignment of the cells and matrix along the longitudinal axis, then stabilized through plastic compression to generate an anisotropic cellular collagen sheet (Georgiou et al. 2013). EngNT has subsequently been shown to support nerve regeneration both *in vitro* and *in vivo* using various different cell options (Georgiou et al. 2015; Sanen

et al. 2017; O'Rourke et al. 2018). Siriwardane et al. described a new way to create collagen scaffolds with spatially oriented and uniform fibers and enhanced biocompatibility via wet spinning. These were shown to successfully support the survival of dorsal root ganglion neurons and Schwann cells which adhered to the scaffolds and migrated along the surface of fibers (Siriwardane et al. 2014). Antman-Passig and Shefi embedded superparamagnetic iron oxide nanoparticles in the collagen matrix and established a method that allowed them to control fibril alignment dynamically and remotely. This enables the in situ control of scaffold orientation which can be tailored to the morphological characteristics of the injury site (Antman-Passig and Shefi 2016).

Multichannel NGCs

Efforts have also been made to integrate microscale tubular collagen structures inside the lumen of conduits and create multichannel NGCs. Bozkurt et al. demonstrated that Schwann cells seeded on longitudinally orientated, freeze-dried, and collagen/elastin scaffolds could migrate through the microchannels, and eventually form cellular columns that resemble the bands of Büngner (Bozkurt et al. 2009). Hu et al. (2009) described a novel NGC composed of collagen and chitosan, in which the inner channel dimensions (25–55 μm) approached those of the basal lamina microchannels in nerve tissue. Upon implantation in a 15 mm rat sciatic nerve defect, the device, which was not seeded with therapeutic cells, supported nerve regeneration and functional recovery (Hu et al. 2009). Although this and other multichannel NGCs (Yao et al. 2010b; Huang et al. 2018) were shown to provide sufficient guidance for the regeneration of severed axons, there are still doubts about their likely efficacy in clinical practice, especially since their increased structural complexity could be linked to reduced permeability and flexibility (de Ruiter et al. 2008).

NGC Modifications and Synergy with Other Nerve Regeneration Therapies

Another key component of collagen NGCs that can be manipulated and optimized is the interaction between cells and the substrate, which can greatly affect several regenerative processes. One relevant strategy is to introduce chemical and biological modifications on the inner surface of NGCs and establish a more biologically appealing milieu (Li et al. 2020). For instance, guided neuronal migration and axonal regeneration can be achieved by functionalizing collagen scaffolds with cell adhesion motifs or myelin-inhibitory molecules (Rafiuddin Ahmed and Jayakumar 2003; Yao et al. 2010a; Li et al. 2015a).

Previous studies have also reported the delivery of several neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and glial cell line-derived neurotrophic factor (GDNF) from collagen NGCs. One of the simplest approaches is based on incorporating them in the lumen filler in free form (Utlely et al. 1996; Ho et al. 1998) or entrapped in nanoparticles/microspheres for greater control over their release and distribution (Langert and Brey 2018). Madduri et al. developed collagen tubes that allowed the dual delivery of synergistically acting GDNF and NGF to enhance function and axonal myelination (Madduri et al. 2010). Ma et al. immobilized GDNF in lyophilized collagen conduits via chemical

conjugation which resulted in a sustained release for more than 32 days. The artificial nerve conduit was then used to examine regeneration across an 8 mm rat facial nerve defect. Based on functional and morphological analysis, the GDNF-anchored group showed significant axonal regeneration, yielding results that approached those of the autograft (Ma et al. 2018). Vascular endothelial growth factor (VEGF) is another growth factor that has a vital role in angiogenesis and exerts positive effects on nerve regeneration (Andreone et al. 2015; Muangsant et al. 2018). Local administration of VEGF has been shown to lead to earlier functional recovery and improved nerve morphology after nerve transection (Mohammadi et al. 2013). Therefore, preliminary studies have explored the use of NGF and VEGF-loaded magnetic nanoparticles for time- and space-controlled release of growth factors inside a NGC (Giannaccini et al. 2017).

Spatial patterning of growth factors from the proximal to the distal end can also help to guide regenerating neuronal growth cones via chemotaxis (Mortimer et al. 2008). Li et al. functionalized electrospun collagen mats produced via electrohydrodynamic jet printing with stromal cell-derived factor-1 α (SDF1 α) to form chemokine gradients. The resulting hybrid conduits were found to exert strong chemotactic effects on neuronal stem cells (Li et al. 2015b). An alternative approach is to control the seeding pattern of cells along the conduit, which have been genetically modified to be capable of producing high levels of growth factors (Sun et al. 2019).

Finally, with the identification of more PNS molecular targets that could enable purposeful pharmacological interventions, encapsulation of small molecules and drugs into NGCs is becoming more popular. Ai et al. used a collagen hydrogel containing insulin in nanoparticulate form to treat a rat sciatic nerve crush injury (Ai et al. 2019). Compared to empty collagen NGCs, animals treated with the composite hydrogels displayed significantly improved outcomes in all postoperative tests including sciatic functional index, hot plate latency, and compound muscle action potential amplitude. Sayanagi et al. examined the effects of combination therapy using an electrospun sheet loaded with methylcobalamin and a tube filled with collagen sponge in a rat model of sciatic nerve injury. Incorporation of methylcobalamin, an active form of vitamin B12, significantly accelerated the recovery of sensory and motor function (Sayanagi et al. 2020).

2.3 Clinically Approved Engineered Collagen Grafts for Nerve Regeneration

Today, the use of artificial NGCs is a clinically approved alternative to the “gold standard” autograft repair in many situations (di Summa et al. 2014; Sanen et al. 2017; Mozafari et al. 2018). Twenty years since the regulatory approval of the first device for PNI repair, collagen remains the most popular natural biomaterial in clinical nerve regeneration (Chrzęszcz et al. 2018) (see Table 3). Due to the limited variety of available devices, clinical experience and reports regarding postoperative

Table 3 Commercially available and FDA-approved collagen nerve conduits

Product name	Biomaterial	Dimensions	Degradation time	Company	FDA clearance date
<i>Avance nerve graft</i>	Decellularized ECM derived from donated cadaveric nerve	L = 15, 30, 50, 70 mm D = 1–5 mm	Nonabsorbable	AxoGen Inc., Alachua, FL	
<i>NeuraGen</i>	Collagen type I	L = 20, 30 mm D = 1.5–7 mm	36–48 months	Integra LifeSciences Corp., Plainsboro, NJ	2001
<i>NeuroFlex</i>	Collagen type I	L = 25 mm D = 2–6 mm	4–8 months	Collagen Matrix Inc., Oakland, NJ	2001
<i>NeuroMatrix</i>	Collagen type I	L = 25 mm D = 2–6 mm	4–8 months	Collagen Matrix Inc., Oakland, NJ	2001
<i>AxoGuard Nerve Connector</i>	Porcine small intestinal submucosa (SIS)	L = 10, 15 mm D = 1.5–7 mm	Nonabsorbable	AxoGen Inc., Alachua, FL	2003
<i>NeuraWrap</i>	Collagen type I	L = 20, 40 mm D = 3–10 mm	36–48 months	Integra Life Sciences Corp., Plainsboro, NJ	2004
<i>NeuroMend</i>	Collagen type I	L = 25, 50 mm D = 4, 6, 12 mm	4–8 months	Collagen Matrix Inc., Oakland, NJ	2006
<i>Neuragen 3D Nerve Matrix</i>	Collagen type I and glycosaminoglycan	L < 63.5 mm D = 1.5–7 mm	9–12 months	Integra LifeSciences Corp., Plainsboro, NJ	2014
<i>Nerbridge</i>	Polyglycolic acid and collagen type I and III	L = 25 mm D = 0.5–4 mm	3–4 months	TOYOBO CO., LTD	2016

efficacy are mostly based on the use of hollow NGCs, nerve wraps, and decellularized collagen allografts.

First-generation NGCs were based on designing a support structure that would guide outgrowth of the transected nerve. Initial clinical studies, investigating the efficiency of collagen tubes in nerve repair, reported promising results, with functional improvement and recovery of sensory function ranging from fair to excellent (Taras et al. 2005, 2011; Lohmeyer et al. 2007; Bushnell et al. 2008; Wangenstein and Kalliainen 2009; Boeckstyns et al. 2013). Collagen conduits were also found to be advantageous over nonresorbable synthetic ones, as the latter were associated with several complications. They tended to be more rigid and could provoke fibrosis and compression syndromes that hampered nerve regeneration and often required secondary surgery for their removal (Merle et al. 1989; Lundborg et al. 2004).

Despite their encouraging success, still only a small fraction of PNI patients could benefit from the commercially available collagen NGCs as they are unable to bridge large nerve defects (>20 mm) and repair complex injuries (Deal et al. 2012). Moreover, the rapid degradation rates of certain collagen conduits (as early as 4 months) have raised the concern they may not provide a stable structural support for sufficient time (Liodaki et al. 2013). These observations emphasized the need for improved structures with physical guidance and biochemical cues that could enhance their repair capacity.

As a result, later efforts were focused on mimicking the architecture of nerve fascicles by integrating microtubes and establishing a more growth permissive environment by incorporating bioactive compounds and therapeutic cells within the conduits. Rigorous research has yielded advanced NGCs with innovative design features and enhanced functionality. NeuraGen[®] 3D Nerve Guide Matrix is the first regulatory-approved device with a luminal filler composed of collagen and glycosaminoglycan (chondroitin-6-sulfate). Saeki et al. investigated the efficiency of a novel collagen NGC with longitudinal filaments in treating 2–30 mm sensory nerve defects. They reported results comparable to autologous nerve grafting, with 75% rate of recovery during the first 12 months (Saeki et al. 2018). However, despite extensive preclinical testing, clinical trials assessing the safety and efficiency of more advanced collagen NGCs remain scarce. To date, relatively little is known about how more complex nerve conduits perform when implanted in humans and how well they can encourage regeneration compared to the gold standard autograft and simpler NGCs.

Finally, considerable commercial and clinical interest has focused recently on collagen ECM scaffolds obtained by decellularizing native tissues. A prominent example are processed nerve allografts, whose neuroregenerative effects have been reported to be inferior to autografts but superior to simple NGCs and empty tubes (Cho et al. 2012; Rinker et al. 2017). Processing the allografts offers an attractive means of circumventing some of the limitations associated with the use of cadaveric tissue, since they provide a suitable substrate for nerve regeneration without the need for systemic immunosuppression. Previous clinical studies report that processed nerve allografts are safe and can be used for the reconstruction of peripheral nerve defects up to 70 mm in length (Guo et al. 2013; Fleming et al. 2014; Zuniga 2015;

Means Jr et al. 2016). However, the majority of the outcomes reported are limited to sensory function. A recent observational study of 22 cases suggested that processed nerve allografts can help 73% of treated patients to regain meaningful motor recovery, with overall results comparable to autografts (Safa et al. 2019). Despite their advantages, the *in vivo* performance and efficacy of processed allografts remains poorer than the autograft, possibly due to low repopulation by Schwann cells (Saheb-Al-Zamani et al. 2013) and increased scarring and fibrosis (Kehoe et al. 2012).

3 Conclusions

Collagen has been pivotal in the development of nerve repair conduits, providing clinical grade resorbable tubes, decellularized tissue grafts, and contributing key components to more sophisticated replacement tissues containing architecture, factors, and living cells (Fig. 2). The clinical translation of NeuraGen® 3D Nerve Guide Matrix® is an important recent example, and numerous emerging nerve repair technologies in preclinical development use collagen as major biomaterial component. Despite limitations associated with the use of animal-derived materials in human applications, collagen remains one of the most popular biomaterials in nerve tissue engineering. Favorable biological integration, abundance in natural nerve ECM, ease of extraction, and versatility as a biomaterial underpin the popularity of collagen as a substrate for tissue engineering. Synthetic alternatives to

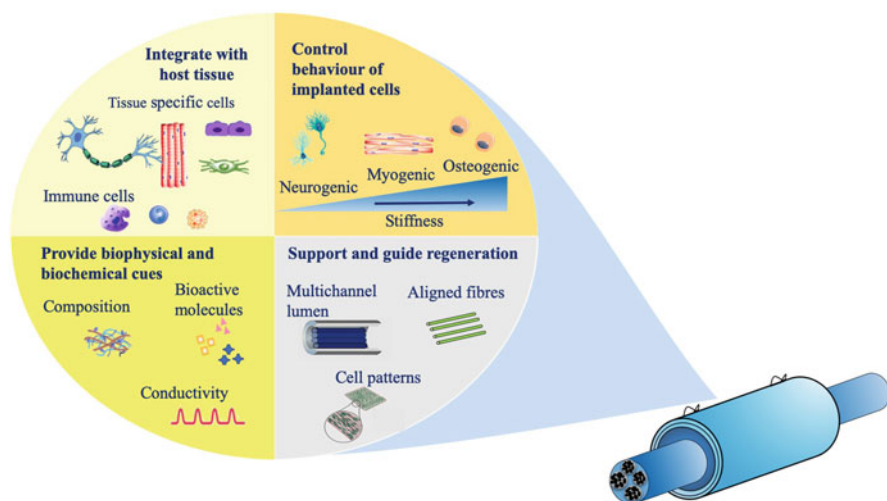


Fig. 2 Collagen as a major biomaterial component in nerve tissue engineering. Sophisticated combinatorial designs of NGCs use collagen as the basis for favorable integration and provision of regenerative chemical and physical cues that control behavior of implanted cells and support and guide regeneration

collagen would potentially overcome challenges associated with the reliance on natural sources, but to date, these have not been developed sufficiently to offer an attractive enough replacement in many nerve tissue engineering applications.

References

- Aamodt JM, Grainger DW (2016) Extracellular matrix-based biomaterial scaffolds and the host response. *Biomaterials* 86:68–82. <https://doi.org/10.1016/j.biomaterials.2016.02.003>
- Ai A, Behforouz A, Ehterami A, Sadeghvaziri N, Jalali S, Farzamfar S, Yousefbeigi A, Ai A, Goodarzi A, Salehi M, Ai J (2019) Sciatic nerve regeneration with collagen type I hydrogel containing chitosan nanoparticle loaded by insulin. *Int J Polym Mater Polym Biomater* 68:1133–1141. <https://doi.org/10.1080/00914037.2018.1534114>
- Andreone BJ, Lacoste B, Gu C (2015) Neuronal and vascular interactions. *Annu Rev Neurosci* 38:25–46. <https://doi.org/10.1146/annurev-neuro-071714-033835>
- Ansselin AD, Fink T, Davey DF (1997) Peripheral nerve regeneration through nerve guides seeded with adult Schwann cells. *Neuropathol Appl Neurobiol* 23:387–398. <https://doi.org/10.1111/j.1365-2990.1997.tb01313.x>
- Antman-Passig M, Shefi O (2016) Remote magnetic orientation of 3D collagen hydrogels for directed neuronal regeneration. *Nano Lett* 16:2567–2573. <https://doi.org/10.1021/acs.nanolett.6b00131>
- Archibald SJ, Krarup C, Shefner J, Li S-T, Madison RD (1991) A collagen-based nerve guide conduit for peripheral nerve repair: an electrophysiological study of nerve regeneration in rodents and nonhuman primates. *J Comp Neurol* 306:685–696. <https://doi.org/10.1002/cne.903060410>
- Archibald SJ, Shefner J, Krarup C, Madison RD (1995) Monkey median nerve repaired by nerve graft or collagen nerve guide tube. *J Neurosci* 15:4109LP–4123LP. <https://doi.org/10.1523/JNEUROSCI.15-05-04109.1995>
- Asher DM (1999) The transmissible spongiform encephalopathy agents: concerns and responses of United States regulatory agencies in maintaining the safety of biologics. *Dev Biol Stand* 100:103–118
- Barros CS, Franco SJ, Müller U (2011) Extracellular matrix: functions in the nervous system. *Cold Spring Harb Perspect Biol* 3:1–24. <https://doi.org/10.1101/cshperspect.a005108>
- Bartlett RD, Eleftheriadou D, Evans R, Choi D, Phillips JB (2020) Mechanical properties of the spinal cord and brain: comparison with clinical-grade biomaterials for tissue engineering and regenerative medicine. *Biomaterials* 258:120303. <https://doi.org/10.1016/j.biomaterials.2020.120303>
- Beck K, Brodsky B (1998) Supercoiled protein motifs: the collagen triple-helix and the α -helical coiled coil. *J Struct Biol* 122:17–29
- Bella J, Hulmes DJS (2017) Fibrillar collagens BT. In: Parry DAD, Squire JM (eds) *Fibrous proteins: structures and mechanisms*. Springer International Publishing, Cham, pp 457–490
- Boeckstyns MEH, Sørensen AI, Viñeta JF, Rosén B, Navarro X, Archibald SJ, Valss-Solé J, Moldovan M, Krarup C (2013) Collagen conduit versus microsurgical neuroorrhaphy: 2-year follow-up of a prospective, blinded clinical and electrophysiological multicenter randomized, controlled trial. *J Hand Surg Am* 38:2405–2411. <https://doi.org/10.1016/j.jhsa.2013.09.038>
- Bozkurt A, Deumens R, Beckmann C, Olde Damink L, Schügner F, Heschel I, Sellhaus B, Weis J, Jahnhen-Dechent W, Brook GA, Pallua N (2009) In vitro cell alignment obtained with a Schwann cell enriched microstructured nerve guide with longitudinal guidance channels. *Biomaterials* 30:169–179. <https://doi.org/10.1016/j.biomaterials.2008.09.017>
- Bozkurt A, Lassner F, O'Dey D, Deumens R, Böcker A, Schwendt T, Janzen C, Suschek CV, Tolba R, Kobayashi E, Sellhaus B, Tholl S, Eummelen L, Schügner F, Olde Damink L, Weis J, Brook GA, Pallua N (2012) The role of microstructured and interconnected pore channels in

- a collagen-based nerve guide on axonal regeneration in peripheral nerves. *Biomaterials* 33:1363–1375. <https://doi.org/10.1016/j.biomaterials.2011.10.069>
- Brown BN, Freund JM, Han L, Rubin JP, Reing JE, Jeffries EM, Wolf MT, Tottey S, Barnes CA, Ratner BD, Badyalak SF (2010) Comparison of three methods for the derivation of a biologic scaffold composed of adipose tissue extracellular matrix. *Tissue Eng Part C Methods* 17:411–421. <https://doi.org/10.1089/ten.tec.2010.0342>
- Browne S, Zeugolis DI, Pandit A (2013) Collagen: finding a solution for the source. *Tissue Eng Part A* 19:1491–1494. <https://doi.org/10.1089/ten.TEA.2012.0721>
- Bulleid, NJ, John DC, and Kadler KE (2000) Recombinant expression systems for the production of collagen. *Biochem Soc Trans* 28(4):350–353. <https://doi.org/10.1042/bst0280350>
- Bushnell BD, McWilliams AD, Whitener GB, Messer TM (2008) Early clinical experience with collagen nerve tubes in digital nerve repair. *J Hand Surg Am* 33:1081–1087. <https://doi.org/10.1016/j.jhsa.2008.03.015>
- Carri NG, Rubin K, Gullberg D, Ebendal T (1992) Neuritogenesis on collagen substrates. Involvement of integrin-like matrix receptors in retinal fibre outgrowth on collagen. *Int J Dev Neurosci* 10:393–405. [https://doi.org/10.1016/0736-5748\(92\)90029-Y](https://doi.org/10.1016/0736-5748(92)90029-Y)
- Cassel JM, Kanagy JR (1949) Studies on the purification of collagen. *J Res Natl Bur Stand* 42:557–565
- Castilla-Casadio DA, Rivera-Martínez CA, Quiñones-Colón BA, Almodóvar J (2017) Electrospun collagen scaffolds BT. In: Almodovar J (ed) *Electrospun biomaterials and related technologies*. Springer International Publishing, Cham, pp 21–55
- Ceballos D, Navarro X, Dubey N, Wendelschafer-Crabb G, Kennedy WR, Tranquillo RT (1999) Magnetically aligned collagen gel filling a collagen nerve guide improves peripheral nerve regeneration. *Exp Neurol* 158:290–300. <https://doi.org/10.1006/exnr.1999.7111>
- Chattopadhyay S, Raines RT (2014) Review collagen-based biomaterials for wound healing. *Biopolymers* 101:821–833. <https://doi.org/10.1002/bip.22486>
- Cheema U, Brown RA (2013) Rapid fabrication of living tissue models by collagen plastic compression: understanding three-dimensional cell matrix repair in vitro. *Adv Wound Care* 2:176–184. <https://doi.org/10.1089/wound.2012.0392>
- Chen F-M, Liu X (2016) Advancing biomaterials of human origin for tissue engineering. *Prog Polym Sci* 53:86–168. <https://doi.org/10.1016/j.progpolymsci.2015.02.004>
- Chen P, Cescon M, Bonaldo P (2015) The role of collagens in peripheral nerve myelination and function. *Mol Neurobiol* 52:216–225. <https://doi.org/10.1007/s12035-014-8862-y>
- Cheng X, Gurkan UA, Dehen CJ, Tate MP, Hillhouse HW, Simpson GJ, Akkus O (2008) An electrochemical fabrication process for the assembly of anisotropically oriented collagen bundles. *Biomaterials* 29:3278–3288. <https://doi.org/10.1016/j.biomaterials.2008.04.028>
- Cho MS, Rinker BD, Weber RV, Chao JD, Ingari JV, Brooks D, Buncke GM (2012) Functional outcome following nerve repair in the upper extremity using processed nerve allograft. *J Hand Surg Am* 37:2340–2349. <https://doi.org/10.1016/j.jhsa.2012.08.028>
- Chrzyszcz P, Derbisz K, Suszyński K, Miodoński J, Trybulski R, Lewin-Kowalik J, Marcol W (2018) Application of peripheral nerve conduits in clinical practice: a literature review. *Neurol Neurochir Pol* 52:427–435. <https://doi.org/10.1016/j.pjnns.2018.06.003>
- Cockerham K, Hsu VJ (2009) Collagen-based dermal fillers: past, present, future. *Facial Plast Surg* 25:106–113
- Cooper A (1970) Thermodynamic studies of the assembly in vitro of native collagen fibrils. *Biochem J* 118:355–365
- Copes F, Pien N, Van Vlierberghe S, Boccafroschi F, Mantovani D (2019) Collagen-based tissue engineering strategies for vascular medicine. *Front Bioeng Biotechnol* 7:166
- Cowan PM, McGavin S, North ACT (1955) The polypeptide chain configuration of collagen. *Nature* 176:1062–1064. <https://doi.org/10.1038/1761062a0>
- Crapo PM, Medberry CJ, Reing JE, Tottey S, van der Merwe Y, Jones KE, Badyalak SF (2012) Biologic scaffolds composed of central nervous system extracellular matrix. *Biomaterials* 33:3539–3547. <https://doi.org/10.1016/j.biomaterials.2012.01.044>

- Cunniffe GM, O'Brien FJ (2011) Collagen scaffolds for orthopedic regenerative medicine. *JOM* 63:66. <https://doi.org/10.1007/s11837-011-0061-y>
- Davidenko N, Gibb T, Schuster C, Best SM, Campbell JJ, Watson CJ, Cameron RE (2012) Biomimetic collagen scaffolds with anisotropic pore architecture. *Acta Biomater* 8:667–676. <https://doi.org/10.1016/j.actbio.2011.09.033>
- Davidenko N, Schuster CF, Bax DV, Raynal N, Farndale RW, Best SM, Cameron RE (2015) Control of crosslinking for tailoring collagen-based scaffolds stability and mechanics. *Acta Biomater* 25:131–142. <https://doi.org/10.1016/j.actbio.2015.07.034>
- Davison-Kotler E, Marshall WS, García-Gareta E (2019) Sources of collagen for biomaterials in skin wound healing. *Bioengineering* 6:1–15. <https://doi.org/10.3390/bioengineering6030056>
- de Ruiter GC, Onyeneho IA, Liang ET, Moore MJ, Knight AM, Malessy MJA, Spinner RJ, Lu L, Currier BL, Yaszemski MJ, Windebank AJ (2008) Methods for in vitro characterization of multi-channel nerve tubes. *J Biomed Mater Res Part A* 84A:643–651. <https://doi.org/10.1002/jbm.a.31298>
- Deal ND, Griffin JW, Hogan MV (2012) Nerve conduits for nerve repair or reconstruction. *JAAOS – J Am Acad Orthop Surg* 20:63
- Dewle A, Pathak N, Rakshasmare P, Srivastava A (2020) Multifarious fabrication approaches of producing aligned collagen scaffolds for tissue engineering applications. *ACS Biomater Sci Eng* 6:779–797. <https://doi.org/10.1021/acsbiomaterials.9b01225>
- di Summa PG, Kingham PJ, Campisi CC, Raffoul W, Kalbermatten DF (2014) Collagen (NeuraGen®) nerve conduits and stem cells for peripheral nerve gap repair. *Neurosci Lett* 572:26–31. <https://doi.org/10.1016/j.neulet.2014.04.029>
- Dong B, Arnoult O, Smith ME, Wnek GE (2009) Electrospinning of collagen nanofiber scaffolds from benign solvents. *Macromol Rapid Commun* 30:539–542. <https://doi.org/10.1002/marc.200800634>
- Ehrmann RL, Gey GO (1956) The growth of cells on a transparent gel of reconstituted rat-tail collagen2. *JNCI J Natl Cancer Inst* 16:1375–1403. <https://doi.org/10.1093/jnci/16.6.1375>
- Engler AJ, Sen S, Sweeney HL, Discher DE (2006) Matrix elasticity directs stem cell lineage specification. *Cell* 126:677–689. <https://doi.org/10.1016/j.cell.2006.06.044>
- Fauzi MB, Lokanathan Y, Aminuddin BS, Ruszymah BHI, Chowdhury SR (2016) Ovine tendon collagen: extraction, characterisation and fabrication of thin films for tissue engineering applications. *Mater Sci Eng C* 68:163–171. <https://doi.org/10.1016/j.msec.2016.05.109>
- Fernández-Pérez J, Ahearne M (2019) The impact of decellularization methods on extracellular matrix derived hydrogels. *Sci Rep* 9:14933. <https://doi.org/10.1038/s41598-019-49575-2>
- Fleming ME, Bharmal H, Valerio I (2014) Regenerative medicine applications in combat casualty care. *Regen Med* 9:179–190. <https://doi.org/10.2217/rme.13.96>
- Frantz C, Stewart KM, Weaver VM (2010) The extracellular matrix at a glance. *J Cell Sci* 123:4195–4200. <https://doi.org/10.1242/jcs.023820>
- Franzke C-W, Tasanen K, Schumann H, Bruckner-Tuderman L (2003) Collagenous transmembrane proteins: collagen XVII as a prototype. *Matrix Biol* 22:299–309. [https://doi.org/10.1016/S0945-053X\(03\)00051-9](https://doi.org/10.1016/S0945-053X(03)00051-9)
- Fratzl P (2008) Collagen: Structure and Mechanics, an Introduction. In: Fratzl P. (eds) Collagen. Springer, Boston. https://doi.org/10.1007/978-0-387-73906-9_1
- Friedemann M, Kalbitzer L, Franz S, Moeller S, Schnabelrauch M, Simon J-C, Pompe T, Franke K (2017) Instructing human macrophage polarization by stiffness and glycosaminoglycan functionalization in 3D collagen networks. *Adv Healthc Mater* 6:1600967. <https://doi.org/10.1002/adhm.201600967>
- Gelse K, Pöschl E, Aigner T (2003) Collagens – structure, function, and biosynthesis. *Adv Drug Deliv Rev* 55:1531–1546. <https://doi.org/10.1016/j.addr.2003.08.002>
- Georges PC, Miller WJ, Meaney DF, Sawyer ES, Janney PA (2006) Matrices with compliance comparable to that of brain tissue select neuronal over glial growth in mixed cortical cultures. *Biophys J* 90:3012–3018. <https://doi.org/10.1529/biophysj.105.073114>
- Georgiou M, Bunting SCJ, Davies HA, Loughlin AJ, Golding JP, Phillips JB (2013) Engineered neural tissue for peripheral nerve repair. *Biomaterials* 34:7335–7343. <https://doi.org/10.1016/j.biomaterials.2013.06.025>

- Georgiou M, Golding JP, Loughlin AJ, Kingham PJ, Phillips JB (2015) Engineered neural tissue with aligned, differentiated adipose-derived stem cells promotes peripheral nerve regeneration across a critical sized defect in rat sciatic nerve. *Biomaterials* 37:242–251. <https://doi.org/10.1016/j.biomaterials.2014.10.009>
- Giannaccini M, Calatayud MP, Poggetti A, Corbianco S, Novelli M, Paoli M, Battistini P, Castagna M, Dente L, Parchi P, Lisanti M, Cavallini G, Junquera C, Goya GF, Raffa V (2017) Magnetic nanoparticles for efficient delivery of growth factors: stimulation of peripheral nerve regeneration. *Adv Healthc Mater* 6:1601429. <https://doi.org/10.1002/adhm.201601429>
- Gilbert TW (2012) Strategies for tissue and organ decellularization. *J Cell Biochem* 113:2217–2222. <https://doi.org/10.1002/jcb.24130>
- Girton TS, Barocas VH, Tranquillo RT (2002) Confined compression of a tissue-equivalent: collagen fibril and cell alignment in response to anisotropic strain. *J Biomech Eng* 124:568–575. <https://doi.org/10.1115/1.1504099>
- Gómez N, Cuadras J, Butí M, Navarro X (1996) Histologic assessment of sciatic nerve regeneration following resection and graft or tube repair in the mouse. *Restor Neurol Neurosci* 10:187–196. <https://doi.org/10.3233/RNN-1996-10401>
- Gordon MK, Hahn RA (2010) Collagens. *Cell Tissue Res* 339:247–257. <https://doi.org/10.1007/s00441-009-0844-4>
- Gorlov IF, Titov EI, Semenov GV, Slozhenkina MI, Sokolov AY, Omarov RS, Goncharov AI, Zlobina EY, Litvinova EV, Karpenko EV (2018) Collagen from porcine skin: a method of extraction and structural properties. *Int J Food Prop* 21:1031–1042. <https://doi.org/10.1080/10942912.2018.1466324>
- Gouveia RM, Connon CJ (2016) 7 – Collagen scaffolds for corneal regeneration. In: Chirila TV, Harkin D (eds) *Biomaterials and regenerative medicine in ophthalmology*. Woodhead Publishing series in biomaterials, 2nd edn. Woodhead Publishing, Duxford, pp 151–177
- Gregorio I, Braghetta P, Bonaldo P, Cescon M (2018) Collagen VI in healthy and diseased nervous system. *Dis Model Mech* 11:dmm032946. <https://doi.org/10.1242/dmm.032946>
- Grimal S, Puech S, Wagener R, Ventéo S, Carroll P, Fichard-Carroll A (2010) Collagen XXVIII is a distinctive component of the peripheral nervous system nodes of Ranvier and surrounds nonmyelinating glial cells. *Glia* 58:1977–1987. <https://doi.org/10.1002/glia.21066>
- Guler S, Aslan B, Hosseinian P, Aydin HM (2017) Supercritical carbon dioxide-assisted decellularization of aorta and cornea. *Tissue Eng Part C Methods* 23:540–547. <https://doi.org/10.1089/ten.tec.2017.0090>
- Guo C, Kaufman LJ (2007) Flow and magnetic field induced collagen alignment. *Biomaterials* 28:1105–1114. <https://doi.org/10.1016/j.biomaterials.2006.10.010>
- Guo Y, Chen G, Tian G, Tapia C (2013) Sensory recovery following decellularized nerve allograft transplantation for digital nerve repair. *J Plast Surg Hand Surg* 47:451–453. <https://doi.org/10.3109/2000656X.2013.778862>
- Gutsmann T, Fantner GE, Kindt JH, Venturoni M, Danielsen S, Hansma PK (2004) Force spectroscopy of collagen fibers to investigate their mechanical properties and structural organization. *Biophys J* 86:3186–3193. [https://doi.org/10.1016/S0006-3495\(04\)74366-0](https://doi.org/10.1016/S0006-3495(04)74366-0)
- Harris JR, Soliakov A, Lewis RJ (2013) In vitro fibrillogenesis of collagen type I in varying ionic and pH conditions. *Micron* 49:60–68. <https://doi.org/10.1016/j.micron.2013.03.004>
- Haugh MG, Murphy CM, O'Brien FJ (2009) Novel freeze-drying methods to produce a range of collagen–glycosaminoglycan scaffolds with tailored mean pore sizes. *Tissue Eng Part C Methods* 16:887–894. <https://doi.org/10.1089/ten.tec.2009.0422>
- Hirose H, Kiktaguchi T, Tabira T (1993) Neurite promoting activity of collagens on embryonic neurons: decreased effect at the postnatal stage. *Tohoku J Exp Med* 170:207–218
- Ho P-R, Coan GM, Cheng ET, Niell C, Tarn DM, Zhou H, Sierra D, Terris DJ (1998) Repair with collagen tubules linked with brain-derived neurotrophic factor and ciliary neurotrophic factor in a rat sciatic nerve injury model. *Arch Otolaryngol Neck Surg* 124:761–766. <https://doi.org/10.1001/archotol.124.7.761>

- Hoffman-Kim D, Mitchel JA, Bellamkonda RV (2010) Topography, cell response, and nerve regeneration. *Annu Rev Biomed Eng* 12:203–231. <https://doi.org/10.1146/annurev-bioeng-070909-105351>
- Hu X, Huang J, Ye Z, Xia L, Li M, Lv B, Shen X, Luo Z (2009) A novel scaffold with longitudinally oriented microchannels promotes peripheral nerve regeneration. *Tissue Eng Part A* 15:3297–3308. <https://doi.org/10.1089/ten.tea.2009.0017>
- Huang L, Zhu L, Shi X, Xia B, Liu Z, Zhu S, Yang Y, Ma T, Cheng P, Luo K, Huang J, Luo Z (2018) A compound scaffold with uniform longitudinally oriented guidance cues and a porous sheath promotes peripheral nerve regeneration in vivo. *Acta Biomater* 68:223–236. <https://doi.org/10.1016/j.actbio.2017.12.010>
- Hubert T, Grimal S, Carroll P, Fichard-Carroll A (2009) Collagens in the developing and diseased nervous system. *Cell Mol Life Sci* 66:1223–1238
- Hudson TW, Zawko S, Deister C, Lundy S, Hu CY, Lee K, Schmidt CE (2004) Optimized acellular nerve graft is immunologically tolerated and supports regeneration. *Tissue Eng* 10:1641–1651. <https://doi.org/10.1089/ten.2004.10.1641>
- Hulmes DJS (2008) Collagen diversity, synthesis and assembly BT. In: Fratzl P (ed) *Collagen: structure and mechanics*. Springer US, Boston, pp 15–47
- Huzella T (1932) Orientation de la croissance des cultures de tissus sur la trame fibrillaire artificielle coagulée de la solution de collagène. *SAC r Soc Biol Paris* 109:515
- Hwang J, San BH, Turner NJ, White LJ, Faulk DM, Badylak SF, Li Y, Yu SM (2017) Molecular assessment of collagen denaturation in decellularized tissues using a collagen hybridizing peptide. *Acta Biomater* 53:268–278. <https://doi.org/10.1016/j.actbio.2017.01.079>
- Ijima FFA, Van De Graaf RC, Meek MF (2008) The early history of tubulation in nerve repair. *J Hand Surg (European)* 33:581–586. <https://doi.org/10.1177/1753193408091349>
- Ishikawa Y, Bächinger HP (2013) A molecular ensemble in the rER for procollagen maturation. *Biochim Biophys Acta, Mol Cell Res* 1833:2479–2491. <https://doi.org/10.1016/j.bbamcr.2013.04.008>
- Itoh S, Takakuda K, Ichinose S, Kikuchi M, Schinomiya K (2001) A study of induction of nerve regeneration using bioabsorbable tubes. *J Reconstr Microsurg* 17:115–124
- Jank BJ, Xiong L, Moser PT, Guyette JP, Ren X, Cetrulo CL, Leonard DA, Fernandez L, Fagan SP, Ott HC (2015) Engineered composite tissue as a bioartificial limb graft. *Biomaterials* 61: 246–256. <https://doi.org/10.1016/j.biomaterials.2015.04.051>
- Jiang J-P, Liu X-Y, Zhao F, Zhu X, Li X-Y, Niu X-G, Yao Z-T, Dai C, Xu H-Y, Ma K, Chen X-Y, Zhang S (2020) Three-dimensional bioprinting collagen/silk fibroin scaffold combined with neural stem cells promotes nerve regeneration after spinal cord injury. *Neural Regen Res* 15:959–968. <https://doi.org/10.4103/1673-5374.268974>
- Johnson KA, Rogers GJ, Roe SC, Howlett CR, Clayton MK, Milthorpe BK, Schindhelm K (1999) Nitrous acid pretreatment of tendon xenografts cross-linked with glutaraldehyde and sterilized with gamma irradiation. *Biomaterials* 20:1003–1015. [https://doi.org/10.1016/S0142-9612\(98\)90187-9](https://doi.org/10.1016/S0142-9612(98)90187-9)
- Kadler KE, Baldock C, Bella J, Boot-Handford RP (2007) Collagens at a glance. *J Cell Sci* 120:1955LP–1958LP. <https://doi.org/10.1242/jcs.03453>
- Kamranpour NO, Miri AK, James-Bhasin M, Nazhat SN (2016) A gel aspiration-ejection system for the controlled production and delivery of injectable dense collagen scaffolds. *Biofabrication* 8:15018. <https://doi.org/10.1088/1758-5090/8/1/015018>
- Karabekmez FE, Duymaz A, Moran SL (2009) Early clinical outcomes with the use of decellularized nerve allograft for repair of sensory defects within the hand. *Hand* 4:245–249. <https://doi.org/10.1007/s11552-009-9195-6>
- Karsdal M (2019) *Biochemistry of collagens, laminins and elastin: structure, function and biomarkers*. Academic, London
- Kasimir M-T, Rieder E, Seebacher G, Silberhumer G, Wolner E, Weigel G, Simon P (2003) Comparison of different decellularization procedures of porcine heart valves. *Int J Artif Organs* 26:421–427. <https://doi.org/10.1177/039139880302600508>

- Kato YP, Christiansen DL, Hahn RA, Shieh S-J, Goldstein JD, Silver FH (1989) Mechanical properties of collagen fibres: a comparison of reconstituted and rat tail tendon fibres. *Biomaterials* 10:38–42. [https://doi.org/10.1016/0142-9612\(89\)90007-0](https://doi.org/10.1016/0142-9612(89)90007-0)
- Kehoe S, Zhang XF, Boyd D (2012) FDA approved guidance conduits and wraps for peripheral nerve injury: a review of materials and efficacy. *Injury* 43:553–572. <https://doi.org/10.1016/j.injury.2010.12.030>
- Keilhoff G, Stang F, Wolf G, Fansa H (2003) Bio-compatibility of type I/III collagen matrix for peripheral nerve reconstruction. *Biomaterials* 24:2779–2787. [https://doi.org/10.1016/S0142-9612\(03\)00084-X](https://doi.org/10.1016/S0142-9612(03)00084-X)
- Kim J, Li WA, Sands W, Mooney DJ (2014) Effect of pore structure of macroporous poly(Lactide-co-Glycolide) scaffolds on the in vivo enrichment of dendritic cells. *ACS Appl Mater Interfaces* 6:8505–8512. <https://doi.org/10.1021/am501376n>
- Koopmans G, Hasse B, Sinis N (2009) Chapter 19: The role of collagen in peripheral nerve repair. *Int Rev Neurobiol* 87:363–79. [https://doi.org/10.1016/S0074-7742\(09\)87019-0](https://doi.org/10.1016/S0074-7742(09)87019-0)
- Krewson CE, Chung SW, Dai W, Mark Saltzman W (1994) Cell aggregation and neurite growth in gels of extracellular matrix molecules. *Biotechnol Bioeng* 43:555–562. <https://doi.org/10.1002/bit.260430704>
- Kubow KE, Vukmirovic R, Zhe L, Klotzsch E, Smith ML, Gourdon D, Luna S, Vogel V (2015) Mechanical forces regulate the interactions of fibronectin and collagen I in extracellular matrix. *Nat Commun* 6:8026. <https://doi.org/10.1038/ncomms9026>
- Langert KA, Brey EM (2018) Strategies for targeted delivery to the peripheral nerve. *Front Neurosci* 12:887
- Leclerc PG, Norman E, Groutsi F, Coffin R, Mayer U, Pizzey J, Tonge D (2007) Impaired axonal regeneration by isolectin b4-binding dorsal root ganglion neurons *in vitro*. *J Neurosci* 27:1190LP–1199LP. <https://doi.org/10.1523/JNEUROSCI.5089-06.2007>
- Lee P, Lin R, Moon J, Lee LP (2006) Microfluidic alignment of collagen fibers for in vitro cell culture. *Biomed Microdevices* 8:35–41. <https://doi.org/10.1007/s10544-006-6380-z>
- Leipzig ND, Shoichet MS (2009) The effect of substrate stiffness on adult neural stem cell behavior. *Biomaterials* 30:6867–6878. <https://doi.org/10.1016/j.biomaterials.2009.09.002>
- Li J, Gao W (2018) Fabrication and characterization of 3D microtubular collagen scaffolds for peripheral nerve repair. *J Biomater Appl* 33:541–552. <https://doi.org/10.1177/0885328218804338>
- Li Y, Asadi A, Monroe MR, Douglas EP (2009) pH effects on collagen fibrillogenesis in vitro: electrostatic interactions and phosphate binding. *Mater Sci Eng C* 29:1643–1649. <https://doi.org/10.1016/j.msec.2009.01.001>
- Li X, Han J, Zhao Y, Ding W, Wei J, Han S, Shang X, Wang B, Chen B, Xiao Z, Dai J (2015a) Functionalized collagen scaffold neutralizing the myelin-inhibitory molecules promoted neurites outgrowth in vitro and facilitated spinal cord regeneration in vivo. *ACS Appl Mater Interfaces* 7:13960–13971. <https://doi.org/10.1021/acsami.5b03879>
- Li X, Liang H, Sun J, Zhuang Y, Xu B, Dai J (2015b) Electrospun collagen fibers with spatial patterning of SDF1a for the guidance of neural stem cells. *Adv Healthc Mater* 4:1869–1876. <https://doi.org/10.1002/adhm.201500271>
- Li R, Li D, Zhang H, Wang J, Li X, Xiao J (2020) Growth factors-based therapeutic strategies and their underlying signaling mechanisms for peripheral nerve regeneration. *Acta Pharmacol Sin*. <https://doi.org/10.1038/s41401-019-0338-1>
- Lin K, Zhang D, Macedo MH, Cui W, Sarmento B, Shen G (2019) Advanced collagen-based biomaterials for regenerative biomedicine. *Adv Funct Mater* 29:1804943. <https://doi.org/10.1002/adfm.201804943>
- Liodaki E, Bos I, Lohmeyer JA, Senyaman O, Mauss KL, Siemers F, Mailaender P, Stang F (2013) Removal of collagen nerve conduits (NeuraGen) after unsuccessful implantation: focus on histological findings. *J Reconstr Microsurg* 29:517–522
- Liu X, Chen W, Gustafson CT, Miller AL 2nd, Waletzki BE, Yaszemski MJ, Lu L (2015) Tunable tissue scaffolds fabricated by in situ crosslink in phase separation system. *RSC Adv* 5:100824–100833. <https://doi.org/10.1039/C5RA19406G>

- Liu X, Li N, Gong D, Xia C, Xu Z (2018) Comparison of detergent-based decellularization protocols for the removal of antigenic cellular components in porcine aortic valve. *Xenotransplantation* 25:e12380. <https://doi.org/10.1111/xen.12380>
- Lohmeyer J, Zimmermann S, Sommer B, Machens H-G, Lange T, Mailänder P (2007) Überbrückung peripherer Nervendefekte durch den Einsatz von Nervenröhrchen. *Der Chir* 78:142–147. <https://doi.org/10.1007/s00104-006-1269-1>
- Lowe CJ, Reucroft IM, Grota MC, Shreiber DI (2016) Production of highly aligned collagen scaffolds by freeze-drying of self-assembled, fibrillar collagen gels. *ACS Biomater Sci Eng* 2:643–651. <https://doi.org/10.1021/acsbiomaterials.6b00036>
- Lundborg G, Rosen B, Dahlin L, Holmberg J, Rosen I (2004) Tubular repair of the median or ulnar nerve in the human forearm: a 5-year follow-up. *J Hand Surg Am* 29:100–107. <https://doi.org/10.1016/j.jhsb.2003.09.018>
- Ma F, Xu F, Li R, Zheng Y, Wang F, Wei N, Zhong J, Tang Q, Zhu T, Wang Z, Zhu J (2018) Sustained delivery of glial cell-derived neurotrophic factors in collagen conduits for facial nerve regeneration. *Acta Biomater* 69:146–155. <https://doi.org/10.1016/j.actbio.2018.01.001>
- Madduri S, di Summa P, Papaloizos M, Kalbermatten D, Gander B (2010) Effect of controlled co-delivery of synergistic neurotrophic factors on early nerve regeneration in rats. *Biomaterials* 31:8402–8409. <https://doi.org/10.1016/j.biomaterials.2010.07.052>
- Mason S, Phillips JB (2011) An ultrastructural and biochemical analysis of collagen in rat peripheral nerves: the relationship between fibril diameter and mechanical properties. *J Peripher Nerv Syst* 16:261–269. <https://doi.org/10.1111/j.1529-8027.2011.00352.x>
- Means KR Jr, Rinker BD, Higgins JP, Payne SH Jr, Merrell GA, Wilgis EFS (2016) A multicenter, prospective, randomized, pilot study of outcomes for digital nerve repair in the hand using hollow conduit compared with processed allograft nerve. *Hand (N Y)* 11:144–151. <https://doi.org/10.1177/1558944715627233>
- Meng FW, Slivka PF, Dearth CL, Badylak SF (2015) Solubilized extracellular matrix from brain and urinary bladder elicits distinct functional and phenotypic responses in macrophages. *Biomaterials* 46:131–140. <https://doi.org/10.1016/j.biomaterials.2014.12.044>
- Merle M, Lee Dellon A, Campbell JN, Chang PS (1989) Complications from silicon-polymer intubulation of nerves. *Microsurgery* 10:130–133. <https://doi.org/10.1002/micr.1920100213>
- Meyer M (2019) Processing of collagen based biomaterials and the resulting materials properties. *Biomed Eng Online* 18:24. <https://doi.org/10.1186/s12938-019-0647-0>
- Meyer C, Wrobel S, Raimondo S, Rochkind S, Heimann C, Shahar A, Ziv-Polat O, Geuna S, Grothe C, Haastert-Talini K (2016) Peripheral nerve regeneration through hydrogel-enriched chitosan conduits containing engineered Schwann cells for drug delivery. *Cell Transplant* 25:159–182. <https://doi.org/10.3727/096368915X688010>
- Mohammadi R, Ahsan S, Masoumi M, Amini K (2013) Vascular endothelial growth factor promotes peripheral nerve regeneration after sciatic nerve transection in rat. *Chin J Traumatol* 16:323–329. <https://doi.org/10.3760/cma.j.issn.1008-1275.2013.06.001>
- Mortimer D, Fothergill T, Pujic Z, Richards LJ, Goodhill GJ (2008) Growth cone chemotaxis. *Trends Neurosci* 31:90–98
- Mouw JK, Ou G, Weaver VM (2014) Extracellular matrix assembly: a multiscale deconstruction. *Nat Rev Mol Cell Biol* 15:771–785. <https://doi.org/10.1038/nrm3902>
- Mozafari R, Kyrilenko S, Castro MV, Ferreira RS, Barraviera B, Oliveira ALR (2018) Combination of heterologous fibrin sealant and bioengineered human embryonic stem cells to improve regeneration following autogenous sciatic nerve grafting repair. *J Venom Anim Toxins Incl Trop Dis* 24:11. <https://doi.org/10.1186/s40409-018-0147-x>
- Muangsanit P, Shipley RJ, Phillips JB (2018) Vascularization strategies for peripheral nerve tissue engineering. *Anat Rec (Hoboken)* 301:1657–1667. <https://doi.org/10.1002/ar.23919>
- Myllyharju J, Kivirikko KI (2004) Collagens, modifying enzymes and their mutations in humans, flies and worms. *Trends Genet* 20:33–43. <https://doi.org/10.1016/j.tig.2003.11.004>
- Myllyharju J, Lamberg A, Notbohm H, Fietzek PP, Pihlajaniemi T, Kivirikko KI (1997) Expression of wild-type and modified pro- α chains of human type I procollagen in insect cells leads to the

- formation of stable $[\alpha 1(I)]_2\alpha 2(I)$ collagen heterotrimers and $[\alpha 1(I)]_3$ homotrimers but not $[\alpha 2(I)]_3$ homotrimers. *J Biol Chem* 272:21824–21830
- Nageotte J (1927) Sur le caillot artificiel de collagène. *C r Soc Biol Paris* 96:172–174
- Nelson CM, Bissell MJ (2006) Of extracellular matrix, scaffolds, and signaling: tissue architecture regulates development, homeostasis, and cancer. *Annu Rev Cell Dev Biol* 22:287–309. <https://doi.org/10.1146/annurev.cellbio.22.010305.104315>
- Nutton V (2012) *Ancient medicine*. Routledge, London.
- O'Rourke C, Day AGE, Murray-Dunning C, Thanabalasundaram L, Cowan J, Stevanato L, Grace N, Cameron G, Drake RAL, Sinden J, Phillips JB (2018) An allogeneic 'off the shelf' therapeutic strategy for peripheral nerve tissue engineering using clinical grade human neural stem cells. *Sci Rep* 8:2951. <https://doi.org/10.1038/s41598-018-20927-8>
- Oh HH, Ko Y-G, Lu H, Kawazoe N, Chen G (2012) Preparation of porous collagen scaffolds with micropatterned structures. *Adv Mater* 24:4311–4316. <https://doi.org/10.1002/adma.201200237>
- Olsen DR, Leigh SD, Chang R, McMullin H, Ong W, Tai E, Chisholm G, Birk DE, Berg RA, Hitzeman RA, Toman PD (2001) Production of human type I collagen in yeast reveals unexpected new insights into the molecular assembly of collagen trimers. *J Biol Chem* 276:24038–24043
- Orgel JPRO, San Antonio JD, Antipova O (2011) Molecular and structural mapping of collagen fibril interactions. *Connect Tissue Res* 52:2–17. <https://doi.org/10.3109/03008207.2010.511353>
- Osawa T, Ide C (1986) Changes in thickness of collagen fibrils in the endo-and epineurium of the mouse sciatic nerve during development. *Cells Tissues Organs* 125:245–251
- Pang K, Du L, Wu X (2010) A rabbit anterior cornea replacement derived from acellular porcine cornea matrix, epithelial cells and keratocytes. *Biomaterials* 31:7257–7265. <https://doi.org/10.1016/j.biomaterials.2010.05.066>
- Parenteau-Bareil R, Gauvin R, Berthod F (2010) Collagen-based biomaterials for tissue engineering applications. *Materials (Basel)* 3:1863–1887. <https://doi.org/10.3390/ma3031863>
- Patino MG, Neiders ME, Andreana S, Noble B, Cohen RE (2002) Collagen as an implantable material in medicine and dentistry. *J Oral Implantol* 28:220–225. [https://doi.org/10.1563/1548-1336\(2002\)028<0220:CAAIMI>2.3.CO;2](https://doi.org/10.1563/1548-1336(2002)028<0220:CAAIMI>2.3.CO;2)
- Patino MG, Neiders ME, Andreana S, Noble B, Cohen RE (2003) Cellular inflammatory response to porcine collagen membranes. *J Periodontal Res* 38:458–464. <https://doi.org/10.1034/j.1600-0765.2003.00017.x>
- Persikov AV, Ramshaw JAM, Kirkpatrick A, Brodsky B (2000) Amino acid propensities for the collagen triple-helix. *Biochemistry* 39:14960–14967. <https://doi.org/10.1021/bi001560d>
- Phillips JB, Bunting SCJ, Hall SM, Brown RA (2005) Neural tissue engineering: A self-organizing collagen guidance conduit. *Tissue Eng* 11:1611–1617. <https://doi.org/10.1089/ten.2005.11.1611>
- Pins GD, Silver FH (1995) A self-assembled collagen scaffold suitable for use in soft and hard tissue replacement. *Mater Sci Eng C* 3:101–107. [https://doi.org/10.1016/0928-4931\(95\)00109-3](https://doi.org/10.1016/0928-4931(95)00109-3)
- Pixley SK, Hopkins TM, Little KJ, Hom DB (2016) Evaluation of peripheral nerve regeneration through biomaterial conduits via micro-CT imaging. *Laryngoscope Investig Otolaryngol* 1:185–190. <https://doi.org/10.1002/lio2.41>
- Prockop DJ, Fertala A (1998) Inhibition of the self-assembly of collagen I into fibrils with synthetic peptides demonstration that assembly is driven by specific binding sites on the monomers. *J Biol Chem* 273:15598–15604
- Quigley AF, Bulluss KJ, Kyrtzlis ILB, Gilmore K, Mysore T, Schirmer KSU, Kennedy EL, O'Shea M, Truong YB, Edwards SL, Peeters G, Herwig P, Razal JM, Campbell TE, Lowes KN, Higgins MJ, Moulton SE, Murphy MA, Cook MJ, Clark GM, Wallace GG, Kapsa RMI (2013) Engineering a multimodal nerve conduit for repair of injured peripheral nerve. *J Neural Eng* 10:16008. <https://doi.org/10.1088/1741-2560/10/1/016008>
- Rafiuddin Ahmed M, Jayakumar R (2003) Peripheral nerve regeneration in RGD peptide incorporated collagen tubes. *Brain Res* 993:208–216. <https://doi.org/10.1016/j.brainres.2003.08.057>
- Rajan N, Habermehl J, Cote M-F, Doillon CJ, Mantovani D (2006) Preparation of ready-to-use, storable and reconstituted type I collagen from rat tail tendon for tissue engineering applications. *Nat Protoc* 1:2753–2758. <https://doi.org/10.1038/nprot.2006.430>

- Ramachandran GN, Kartha G (1954) Structure of collagen. *Nature* 174:269–270. <https://doi.org/10.1038/174269c0>
- Ramshaw JAM, Shah NK, Brodsky B (1998) Gly-X-Y tripeptide frequencies in collagen: a context for host-guest triple-helical peptides. *J Struct Biol* 122:86–91. <https://doi.org/10.1006/jสบi.1998.3977>
- Ren T, Faust A, van der Merwe Y, Xiao B, Johnson S, Kandakatla A, Gorantla VS, Badyalak SF, Washington KM, Steketee MB (2018) Fetal extracellular matrix nerve wraps locally improve peripheral nerve remodeling after complete transection and direct repair in rat. *Sci Rep* 8:4474. <https://doi.org/10.1038/s41598-018-22628-8>
- Ricard-Blum S (2011) The collagen family. *Cold Spring Harb Perspect Biol* 3:a004978–a004978. <https://doi.org/10.1101/cshperspect.a004978>
- Rich A, Crick F (1955) The structure of collagen. *Nature* 176:915
- Ring C, Hassell J, Halfter W (1996) Expression pattern of collagen IX and potential role in the segmentation of the peripheral nervous system. *Dev Biol* 180:41–53. <https://doi.org/10.1006/dbio.1996.0283>
- Rinker B, Zoldos J, Weber RV, Ko J, Thayer W, Greenberg J, Leversedge FJ, Safa B, Buncke G (2017) Use of processed nerve allografts to repair nerve injuries greater than 25 mm in the hand. *Ann Plast Surg* 78:S292
- Robins SP (2007) Biochemistry and functional significance of collagen cross-linking. *Biochem Soc Trans* 35:849–852. <https://doi.org/10.1042/BST0350849>
- Rodrigues CVM, Serricella P, Linhares ABR, Guerdes RM, Borojevic R, Rossi MA, Duarte MEL, Farina M (2003) Characterization of a bovine collagen–hydroxyapatite composite scaffold for bone tissue engineering. *Biomaterials* 24:4987–4997. [https://doi.org/10.1016/S0142-9612\(03\)00410-1](https://doi.org/10.1016/S0142-9612(03)00410-1)
- Roeder BA, Kokini K, Sturgis JE, Robinson JP, Voytik-Harbin SL (2002) Tensile mechanical properties of three-dimensional type I collagen extracellular matrices with varied microstructure. *J Biomech Eng* 124:214–222. <https://doi.org/10.1115/1.1449904>
- Rosso G, Liashkovich I, Young P, Röhr D, Shahin V (2017) Schwann cells and neurite outgrowth from embryonic dorsal root ganglions are highly mechanosensitive. *Nanomedicine* 13:493–501. <https://doi.org/10.1016/j.nano.2016.06.011>
- Rutschmann C, Baumann S, Cabalzar J, Luther KB, Hennet T (2014) Recombinant expression of hydroxylated human collagen in *Escherichia coli*. *Appl Microbiol Biotechnol* 98:4445–4455. <https://doi.org/10.1007/s00253-013-5447-z>
- Saeki M, Tanaka K, Imatani J, Okamoto H, Watanabe K, Nakamura T, Gotani H, Ohi H, Nakamura R, Hirata H (2018) Efficacy and safety of novel collagen conduits filled with collagen filaments to treat patients with peripheral nerve injury: a multicenter, controlled, open-label clinical trial. *Injury* 49:766–774. <https://doi.org/10.1016/j.injury.2018.03.011>
- Safa B, Shores JT, Ingari JV, Weber RV, Cho M, Zoldos J, Niaccaras TR, Nesti LJ, Thayer WP, Buncke GM (2019) Recovery of motor function after mixed and motor nerve repair with processed nerve allograft. *Plast Reconstr Surg Glob Open* 7:e2163–e2163. <https://doi.org/10.1097/GOX.00000000000002163>
- Saheb-Al-Zamani M, Yan Y, Farber SJ, Hunter DA, Newton P, Wood MD, Stewart SA, Johnson PJ, Mackinnon SE (2013) Limited regeneration in long acellular nerve allografts is associated with increased Schwann cell senescence. *Exp Neurol* 247:165–177. <https://doi.org/10.1016/j.expneurol.2013.04.011>
- Sanen K, Martens W, Georgiou M, Ameloot M, Lambrichts I, Phillips J (2017) Engineered neural tissue with Schwann cell differentiated human dental pulp stem cells: potential for peripheral nerve repair? *J Tissue Eng Regen Med* 11:3362–3372. <https://doi.org/10.1002/term.2249>
- Sayanagi J, Tanaka H, Ebara M, Okada K, Oka K, Murase T, Yoshikawa H (2020) Combination of electrospun nanofiber sheet incorporating methylcobalamin and PGA-collagen tube for treatment of a sciatic nerve defect in a rat model. *J Bone Joint Surg* 102:245
- Schneider VA, Granato M (2006) The myotomal diwanka (lh3) glycosyltransferase and type XVIII collagen are critical for motor growth cone migration. *Neuron* 50:683–695. <https://doi.org/10.1016/j.neuron.2006.04.024>

- Schuh CMAP, Day AGE, Redl H, Phillips J (2018) An optimized collagen-fibrin blend engineered neural tissue promotes peripheral nerve repair. *Tissue Eng Part A* 24:1332–1340. <https://doi.org/10.1089/ten.tea.2017.0457>
- Seo Y, Jung Y, Kim SH (2018) Decellularized heart ECM hydrogel using supercritical carbon dioxide for improved angiogenesis. *Acta Biomater* 67:270–281. <https://doi.org/10.1016/j.actbio.2017.11.046>
- Seyer JM, Kang AH, Whitaker JN (1977) The characterization of type I and type III collagens from human peripheral nerve. *Biochim Biophys Acta – Protein Struct* 492:415–425. [https://doi.org/10.1016/0005-2795\(77\)90093-9](https://doi.org/10.1016/0005-2795(77)90093-9)
- Shahriari D, Koffler J, Lynam DA, Tuszyński MH, Sakamoto JS (2016) Characterizing the degradation of alginate hydrogel for use in multilumen scaffolds for spinal cord repair. *J Biomed Mater Res Part A* 104:611–619. <https://doi.org/10.1002/jbm.a.35600>
- Shoulders MD, Raines RT (2009) Collagen structure and stability. *Annu Rev Biochem* 78:929–958. <https://doi.org/10.1146/annurev.biochem.77.032207.120833>
- Siironen J, Sandberg M, Vuorinen V, Røytta M (1992) Expression of type I and III collagens and fibronectin after transection of rat sciatic nerve. Reinnervation compared with denervation. *Lab Invest* 67:80–87
- Siriwardane ML, DeRosa K, Collins G, Pfister BJ (2014) Controlled formation of cross-linked collagen fibers for neural tissue engineering applications. *Biofabrication* 6:15012. <https://doi.org/10.1088/1758-5082/6/1/015012>
- Snyder CC (1976) On the history of the suture. *Plast Reconstr Surg* 58:401
- Song E, Yeon Kim S, Chun T, Byun H-J, Lee YM (2006) Collagen scaffolds derived from a marine source and their biocompatibility. *Biomaterials* 27:2951–2961. <https://doi.org/10.1016/j.biomaterials.2006.01.015>
- Stang F, Fansa H, Wolf G, Keilhoff G (2005) Collagen nerve conduits – assessment of biocompatibility and axonal regeneration. *Biomed Mater Eng* 15:3–12
- Stang F, Keilhoff G, Fansa H (2009) Biocompatibility of different nerve tubes. *Materials (Basel)* 2:1480–1507. <https://doi.org/10.3390/ma2041480>
- Stein H, Wilensky M, Tsafrir Y, Rosenthal M, Amir R, Avraham T, Ofir K, Dgany O, Yayon A, Shoseyov O (2009) Production of bioactive, post-translationally modified, heterotrimeric, human recombinant type-I collagen in transgenic tobacco. *Biomacromolecules* 10:2640–2645. <https://doi.org/10.1021/bm900571b>
- Stover DA, Verelli BC (2010) Comparative vertebrate evolutionary analyses of type I collagen: potential of COL1a1 gene structure and intron variation for common bone-related diseases. *Mol Biol Evol* 28:533–542. <https://doi.org/10.1093/molbev/msq221>
- Sun AX, Prest TA, Fowler JR, Brick RM, Gloss KM, Li X, DeHart M, Shen H, Yang G, Brown BN, Alexander PG, Tuan RS (2019) Conduits harnessing spatially controlled cell-secreted neurotrophic factors improve peripheral nerve regeneration. *Biomaterials* 203:86–95. <https://doi.org/10.1016/j.biomaterials.2019.01.038>
- Suzuki E, Fraser RDB, MacRae TP (1980) Role of hydroxyproline in the stabilization of the collagen molecule via water molecules. *Int J Biol Macromol* 2:54–56
- Taras JS, Nanavati V, Steelman P (2005) Nerve conduits. *J Hand Ther* 18:191–197. <https://doi.org/10.1197/jjht.2005.02.012>
- Taras JS, Jacoby SM, Lincoski CJ (2011) Reconstruction of digital nerves with collagen conduits. *J Hand Surg Am* 36:1441–1446. <https://doi.org/10.1016/j.jhsa.2011.06.009>
- Terzi A, Gallo N, Bettini S, Sibillano T, Altamura D, Campa L, Natali ML, Salvatore L, Madaghiele M, De Caro L, Valli L, Sannino A, Giannini C (2019) Investigations of processing-induced structural changes in horse type-I collagen at sub and supramolecular levels. *Front Bioeng Biotechnol* 7:203
- Theocharis AD, Skandalis SS, Gialeli C, Karamanos NK (2016) Extracellular matrix structure. *Adv Drug Deliv Rev* 97:4–27. <https://doi.org/10.1016/j.addr.2015.11.001>
- Thomsen L, Bellemere P, Loubersac T, Gaisne E, Poirier P, Chaise F (2010) Treatment by collagen conduit of painful post-traumatic neuromas of the sensitive digital nerve: a retrospective study of 10 cases. *Chir Main* 29:255–262. <https://doi.org/10.1016/j.main.2010.07.004>

- Toman PD, Chisholm G, McMullin H, Giere LM, Olsen DR, Kovach RJ, Leigh SD, Fong BE, Chang R, Daniels GA, Berg RA, Hitzeman RA (2000) Production of recombinant human type I procollagen trimers using a four-gene expression system in the yeast *Saccharomyces cerevisiae*. *J Biol Chem* 275:23303–23309
- Tukmachiev D, Forostyak S, Koci Z, Zaviskova K, Vackova I, Vyborny K, Sandvig I, Sandvig A, Medberry CJ, Badylak SF, Sykova E, Kubinova S (2016) Injectable extracellular matrix hydrogels as scaffolds for spinal cord injury repair. *Tissue Eng Part A* 22:306–317. <https://doi.org/10.1089/ten.TEA.2015.0422>
- Ushiki T, Ide C (1990) Three-dimensional organization of the collagen fibrils in the rat sciatic nerve as revealed by transmission- and scanning electron microscopy. *Cell Tissue Res* 260:175–184. <https://doi.org/10.1007/BF00297503>
- Uttley DS, Lewin SL, Cheng ET, Verity AN, Sierra D, Terris DJ (1996) Brain-derived neurotrophic factor and collagen tubulization enhance functional recovery after peripheral nerve transection and repair. *Arch Otolaryngol Neck Surg* 122:407–413. <https://doi.org/10.1001/archotol.1996.01890160047009>
- Vader D, Kabla A, Weitz D, Mahadevan L (2009) Strain-induced alignment in collagen gels. *PLoS One* 4:e5902–e5902. <https://doi.org/10.1371/journal.pone.0005902>
- Valentini RF, Aebischer P, Winn SR, Galletti PM (1987) Collagen- and laminin-containing gels impede peripheral nerve regeneration through semipermeable nerve guidance channels. *Exp Neurol* 98:350–356. [https://doi.org/10.1016/0014-4886\(87\)90247-0](https://doi.org/10.1016/0014-4886(87)90247-0)
- van Wachem PB, van Gulik TM, van Luyn MJA, Bleichrodt RP (2001) Collagen-based prostheses for hernia repair BT. In: Bendavid R, Abrahamson J, Arregui ME, Flament JB, Phillips EH (eds) *Abdominal wall hernias: principles and management*. Springer, New York, pp 250–257
- Veit G, Kobbe B, Keene DR, Paulsson M, Koch M, Wagener R (2006) Collagen XXVIII, a novel von Wille brand factor a domain-containing protein with many imperfections in the collagenous domain. *J Biol Chem* 281:3494–3504
- Verdú E, Labrador RO, Rodríguez FJ, Ceballos D, Forés J, Navarro X (2002) Alignment of collagen and laminin-containing gels improve nerve regeneration within silicone tubes. *Restor Neurol Neurosci* 20:169–179
- Wallace DG, Rosenblatt J (2003) Collagen gel systems for sustained delivery and tissue engineering. *Adv Drug Deliv Rev* 55:1631–1649. <https://doi.org/10.1016/j.addr.2003.08.004>
- Wang B, Borazjani A, Tahai M, de Jongh Curry AL, Simionescu DT, Guan J, To F, Elder SH, Liao J (2010) Fabrication of cardiac patch with decellularized porcine myocardial scaffold and bone marrow mononuclear cells. *J Biomed Mater Res Part A* 94A:1100–1110. <https://doi.org/10.1002/jbm.a.32781>
- Wangensteen KJ, Kalliainen LK (2009) Collagen tube conduits in peripheral nerve repair: a retrospective analysis. *Hand* 5:273–277. <https://doi.org/10.1007/s11552-009-9245-0>
- Wangensteen KJ, Kalliainen LK (2010) Collagen tube conduits in peripheral nerve repair: a retrospective analysis. *Hand* 5:273–277
- Whitlock EL, Tuffaha SH, Luciano JP, Yan Y, Hunter DA, Magill CK, Moore AM, Tong AY, Mackinnon SE, Borschel GH (2009) Processed allografts and type I collagen conduits for repair of peripheral nerve gaps. *Muscle Nerve* 39:787–799. <https://doi.org/10.1002/mus.21220>
- Willits RK, Skornia SL (2004) Effect of collagen gel stiffness on neurite extension. *J Biomater Sci Polym Ed* 15:1521–1531. <https://doi.org/10.1163/1568562042459698>
- Wood A, Ogawa M, Portier RJ, Schexnayder M, Shirley M, Losso JN (2008) Biochemical properties of alligator (*Alligator mississippiensis*) bone collagen. *Comp Biochem Physiol Part B Biochem Mol Biol* 151:246–249. <https://doi.org/10.1016/j.cbpb.2008.05.015>
- Xiao T, Baier H (2007) Lamina-specific axonal projections in the zebrafish tectum require the type IV collagen dragnet. *Nat Neurosci* 10:1529–1537. <https://doi.org/10.1038/nn2002>
- Xu H, Xu B, Yang Q, Li X, Ma X, Xia Q, Zhang Y, Zhang C, Wu Y, Zhang Y (2014) Comparison of decellularization protocols for preparing a decellularized porcine annulus fibrosus scaffold. *PLoS One* 9:e86723–e86723. <https://doi.org/10.1371/journal.pone.0086723>
- Yanagawa F, Sugiura S, Kanamori T (2016) Hydrogel microfabrication technology toward three dimensional tissue engineering. *Regen Ther* 3:45–57. <https://doi.org/10.1016/j.reth.2016.02.007>

- Yang C, Hillas PJ, Báez JA, Nokelainen M, Balan J, Tang J, Spiro R, Polarek JW (2004) The application of recombinant human collagen in tissue engineering. *BioDrugs* 18:103–119. <https://doi.org/10.2165/00063030-200418020-00004>
- Yannas IV, Orgill DP, Silver J, Norregaard TV, Zervas NT, Schoene WC (1985) Polymeric template facilitates regeneration of sciatic nerve across 15-mm GAP. In: Transactions of the annual meeting of the society for biomaterials in conjunction with the interna, p 146
- Yao L, Damodaran G, Nikolskaya N, Gorman AM, Windebank A, Pandit A (2010a) The effect of laminin peptide gradient in enzymatically cross-linked collagen scaffolds on neurite growth. *J Biomed Mater Res Part A* 92A:484–492. <https://doi.org/10.1002/jbm.a.32359>
- Yao L, de Ruiter GCW, Wang H, Knight AM, Spinner RJ, Yaszemski MJ, Windebank AJ, Pandit A (2010b) Controlling dispersion of axonal regeneration using a multichannel collagen nerve conduit. *Biomaterials* 31:5789–5797. <https://doi.org/10.1016/j.biomaterials.2010.03.081>
- Zeugolis DI, Khew ST, Yew ESY, Ekaputra AK, Tong YW, Yung L-YL, Huttmacher DW, Sheppard C, Raghunath M (2008) Electro-spinning of pure collagen nano-fibres - just an expensive way to make gelatin? *Biomaterials* 29:2293–2305. <https://doi.org/10.1016/j.biomaterials.2008.02.009>
- Zhang Y, Sun T, Jiang C (2018) Biomacromolecules as carriers in drug delivery and tissue engineering. *Acta Pharm Sin B* 8:34–50. <https://doi.org/10.1016/j.apsb.2017.11.005>
- Zhou J, Fritze O, Schleicher M, Wendel H-P, Schenke-Layland K, Harasztosi C, Hu S, Stock UA (2010) Impact of heart valve decellularization on 3-D ultrastructure, immunogenicity and thrombogenicity. *Biomaterials* 31:2549–2554. <https://doi.org/10.1016/j.biomaterials.2009.11.088>
- Zhu S, Yuan Q, Yin T, You J, Gu Z, Xiong S, Hu Y (2018) Self-assembly of collagen-based biomaterials: preparation, characterizations and biomedical applications. *J Mater Chem B* 6:2650–2676. <https://doi.org/10.1039/C7TB02999C>
- Zhu M, Li W, Dong X, Yuan X, Midgley AC, Chang H, Wang Y, Wang H, Wang K, Ma PX, Wang H, Kong D (2019) In vivo engineered extracellular matrix scaffolds with instructive niches for oriented tissue regeneration. *Nat Commun* 10:4620. <https://doi.org/10.1038/s41467-019-12545-3>
- Zochodne DW (2000) The microenvironment of injured and regenerating peripheral nerves. *Muscle Nerve* 23:S33–S38. [https://doi.org/10.1002/1097-4598\(2000\)999:9<::AID-MUS7>3.0.CO;2-F](https://doi.org/10.1002/1097-4598(2000)999:9<::AID-MUS7>3.0.CO;2-F)
- Zuniga JR (2015) Sensory outcomes after reconstruction of lingual and inferior alveolar nerve discontinuities using processed nerve allograft – a case series. *J Oral Maxillofac Surg* 73: 734–744. <https://doi.org/10.1016/j.joms.2014.10.030>

Part IV

Therapeutic Options for Peripheral Nerve Regeneration

Schwann Cells in Nerve Repair and Regeneration

Kristjan R. Jessen and Rhona Mirsky

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Abstract

After peripheral nerve injury, both Schwann cells and PNS neurons reprogram to new phenotypes that promote repair. Myelin and Remak cells generate repair Schwann cells that support neuronal survival and promote axonal regeneration. Trophic factors, cytokines, and epithelial-mesenchymal transition (EMT) genes are upregulated, myelin genes are downregulated, autophagy is activated, and the cells proliferate, elongate, and branch to form regeneration tracks. This reprogramming has features in common with injury responses in other tissues. The repair Schwann cell phenotype fades with time after injury, and in aged animals the activation of the repair phenotype is subdued. This is a key contributor to poor axonal regeneration through the distal nerve stump after long-term denervation and in aged animals. The connective and epithelial tissues of peripheral nerves are important factors in nerve homeostasis and injury, and the formation of these protective tissues is dependent on developing Schwann cells. It has become clear that among the

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mechanisms that control repair cells are dedicated signals, including c-Jun, Merlin, STAT3, and epigenetic mechanisms that have relatively little or no function in development. In the future, it will be important to learn more about the distinctive signalling pathways that control the performance and long-term maintenance of repair Schwann cells, since this will facilitate the search for ways to manipulate these mechanisms for promoting regeneration.

1 Introduction: The Basics of Regeneration

Peripheral nerves potentially regenerate after damage because the differentiation of PNS neurons and Schwann cells is not fixed, a feature that allows these cells to switch to pro-repair phenotypes after injury. Damaged PNS neurons activate an extensive regeneration program involving hundreds of genes, including a large number of gene regulatory proteins, resulting in a change in neuronal function from that of organizing cell-cell signalling to that of rebuilding an axon. This response has been referred to as the cell body reaction or the signalling to growth mode switch (Blesch et al. 2012; reviewed in Fu and Gordon 1997; Doron-Mandel et al. 2015). Similarly, the myelin and non-myelin (Remak) Schwann cells change expression of some thousands of genes after injury as they convert to a regenerative Schwann cell phenotype, the repair Schwann cell, which is specialized to promote axon regrowth (Figs. 1 and 2).

This plasticity of the PNS contrasts with the CNS, where regenerative mechanisms are poorly developed and axonal regeneration is severely compromised. Thus, injured CNS neurons only have limited capacity to switch to a regenerative mode, and oligodendrocytes serve no helpful function for regeneration distal to injury. Microglia are ineffective at breaking down myelin in contrast to macrophages that invade peripheral nerves, while many aspects of the astrocyte response to injury are hostile to axonal regrowth (reviewed in Vargas and Barres 2007; Bradke et al. 2012; Doron-Mandel et al. 2015; Fawcett and Verhaagen 2018).

Schwann cell reprogramming following injury can be separated into three distinct groups of events (Fig. 2). First, Schwann cells gain a number of properties that promote regeneration (reviewed in Chen et al. 2007; Scheib and Höke 2013; Boerboom et al. 2017; Castelnovo et al. 2017; Jessen and Mirsky 2016, 2019b; Jessen and Arthur-Farraj 2018). This involves upregulation of trophic factors, including GDNF, BDNF, NGF, IGF-1, and other proteins that potentially support neuronal survival and axonal elongation; cell surface molecules such as P75NTR, N-cadherin, and L1; and the transcription factor c-Jun that is an important amplifier of the regeneration-supportive program in repair cells (see Sect. 7). Schwann cells also activate the innate immune response, expressing cytokines such as TNF α , LIF, IL-1 α , and MCP-1 for attracting macrophages, cells that provide signals to enhance vascularization and axon growth. Macrophages also join in with Schwann cells in the phagocytosis of myelin in the later stages of myelin clearance, while Schwann cell initiates myelin clearance by activating autophagy, myelinophagy. Loss of axonal contact prompts Schwann cells, cells that are already elongated along axons, to lengthen still further, achieving two- to threefold their original length,

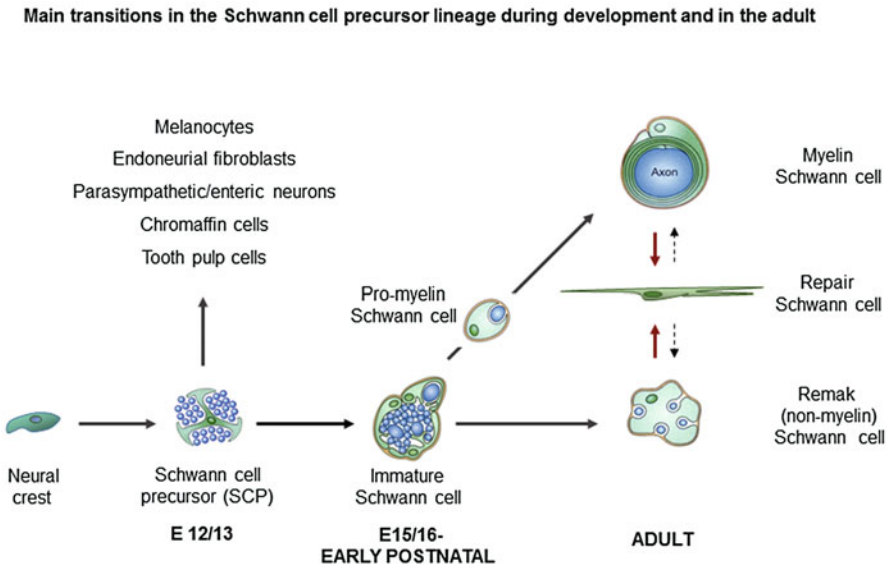


Fig. 1 The main stages in the Schwann cell precursor lineage during development and after injury. Black uninterrupted arrows: normal development. Red arrows: the Schwann cell injury response. Black stippled arrows show the reformation of Remak and myelin cells after regeneration. (With permission from Jessen and Mirsky 2019b)

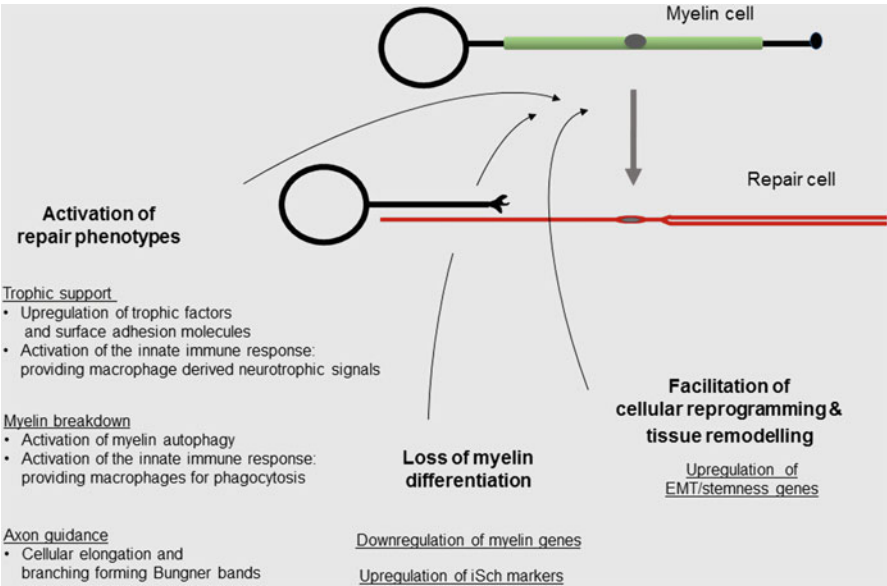


Fig. 2 The Schwann cell injury response. Schwann cell reprogramming after injury can be separated into three main types of event as indicated. For additional details see text

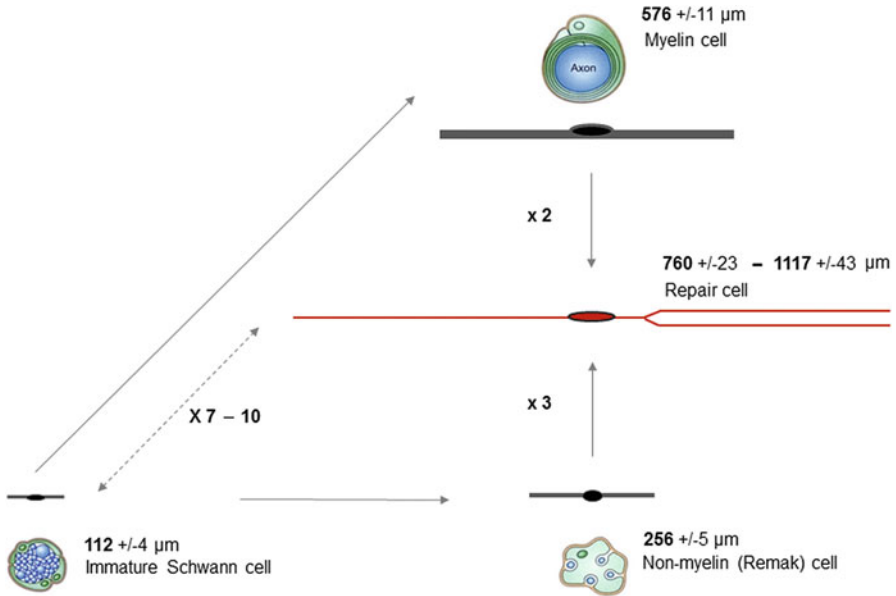


Fig. 3 Schwann cells elongate two- to threefold, and frequently branch, as they transform to repair cells. The diagram shows the length of mouse Schwann cells *in vivo* during development and after injury (Gomez-Sanchez et al. 2017)

and in many cases form long parallel branches, as they arrange in overlapping fashion to form the intertwined cellular columns of the Bungner bands that guide regenerating axons (Fig. 3). Nerve injury also results in a transient wave of Schwann cell proliferation.

The part of the Schwann cell injury response outlined above has often been referred to as activation (Armstrong et al. 2007; Campana 2007; Webber and Zochodne 2010; Allodi et al. 2012). Another main component of the injury response is dedifferentiation, namely, the unwinding of the Schwann cell differentiation that, during development, is induced by axonal signals and results in the emergence of the distinct myelin and Remak cells (reviewed in Chen et al. 2007; Scheib and Höke 2013; Boerboom et al. 2017; Jessen and Mirsky 2016, 2019b; Jessen and Arthur-Farraj 2018). This involves downregulation of myelin-associated proteins including MPZ, MBP, and MAG and periaxin, enzymes involved in cholesterol synthesis, and myelin-related transcription factors such as *Egr2* (*Krox20*). Conversely markers that characterized immature Schwann cells prior to myelination, like GFAP, L1, and P75NTR, reappear. It is likely that in principle comparable, although quantitatively much less impressive, loss of axon-dependent properties takes place in Remak cells after injury, although this has not been closely investigated.

Lastly, accompanying these cellular transformations, and likely facilitating them, Schwann cells in injured nerves activate genes associated with epithelial-mesenchymal transition (EMT) and stemness (Fig. 2) (Arthur-Farraj et al. 2017; Clements et al. 2017; reviewed in Jessen and Arthur-Farraj 2018). In many systems, activation

of these genes is associated with enhanced cellular plasticity, change in differentiation state, and tissue remodeling, all of which are an essential part of the Schwann cell injury response. Enhanced expression of EMT genes is now established as a normal physiological response to injury in numerous tissues (reviewed in Nieto et al. 2016; Skrypek et al. 2017; Jessen and Arthur-Farraj 2018).

The activation of repair supportive features and the loss of myelin differentiation combine to generate a Schwann cell specialized for nerve repair. We therefore refer to this cell as the repair Schwann cell, a cell that differs from other cells in the lineage in a number of obvious ways, including structure, molecular expression, and function (Jessen and Mirsky 2016, 2019b; Jessen and Arthur-Farraj 2018).

The extreme flexibility of Schwann cell differentiation allows these cells at any time to express a phenotype that meets the demands of their immediate environment, whether it be large or small axons in uninjured nerves or degenerating/regenerating axons after injury. One interesting consequence of this plasticity is that the injury response of peripheral nerves does not appear to rely on the presence of standby cells, such as satellite cells in skeletal muscle or NG2 cells in the CNS (Dimou and Gallo 2015; Dumont et al. 2015). Rather, the renowned regeneration potential of peripheral nerves relies on the reprogramming of myelin and Remak cells to repair Schwann cells, in addition to the favorable damage response of peripheral neurons.

2 Schwann Cell Reprogramming Is Related to Injury Responses in Other Tissues

The formation of repair Schwann cells, involving the combination of activation (alternative differentiation) and dedifferentiation, is closely related to injury responses in other tissues. A recent example is the identification of EMT activation in injured Schwann cells mentioned above, since activation of EMT genes is now recognized as a common component of injury responses in many tissues. In a number of other systems also, cells change phenotype in response to injury, forming cells that are specialized to support recovery. We have termed this type of injury response adaptive cellular reprogramming (Jessen et al. 2015a). The common feature of these cell type conversions is the combination of dedifferentiation and alternative differentiation, or activation, to generate new differentiation states, which compensate for injury or promote healing. A classic example is provided by the conversion of pigment epithelium to lens epithelium after eye injury in the newt. This process involves the depigmentation and inhibition of melanogenesis (dedifferentiation) in combination with the gain (activation) of crystallin synthesis to form the transparent lens (reviewed in Shen et al. 2004; Tsonis et al. 2004). In an example from mammals, the conversion of α -cells to β -cells in the pancreatic islets following destruction of β -cells is accomplished by downregulation of glucagon levels (dedifferentiation) and gain (activation) of insulin expression (Thorel et al. 2010; Chera et al. 2014). Other mammalian examples include the conversion of supporting cells to hair cells after ear damage and conversion of fibroblasts to myofibroblasts in skin wounds (reviewed in Jessen et al. 2015a).

Cellular conversions such as those discussed above are often referred to as direct reprogramming (reviewed in Graf and Enver 2009; Sisakhtnezhad and Matin 2012). The application of these concepts to the Schwann cell injury response reflects a more comprehensive understanding than simply using the term dedifferentiation or, alternatively, activation, since these terms refer selectively to only a part of the events that take place as Schwann cells respond to injury (Jessen et al. 2015a).

3 The Repair Schwann Cell Phenotype Emerges Gradually and Fades with Time

Schwann cells distal to an injury site detect axonal interruption within 24 h and before structural axonal degeneration. The molecular mechanisms that initiate these early events and trigger the Schwann cell injury response are largely unknown. The different components of the injury response are not activated synchronously but emerge in a temporal sequence of molecular and morphological changes (reviewed in Jessen and Arthur-Farraj 2018). It is likely that this sequence is controlled by a combination of default programs initiated by a loss of signals from healthy axons and inducible programs driven by injury signals from degenerating axons and invading cells such as macrophages. Some of the earliest events of the injury response relate to activation of the innate immune response, with expression of cytokines such as TNF α and IL-1 β reaching maximum within 1 day after injury and falling sharply to lower levels 2 days later. Myelin autophagy is active at 5 days and decreases subsequently. GDNF expression peaks at about 7 days, while BDNF levels are not maximal until at 2–3 weeks. The transcription factor c-Jun is activated early, but c-Jun protein takes 1–2 weeks to reach maximum levels. Lastly, the Schwann cell elongation triggered by injury continues for at least 4 weeks after loss of axonal contact.

The repair Schwann cell phenotype that emerges through these changes is not stable but fades with time after injury. This, together with a gradual reduction in Schwann cell numbers, is thought to be an important reason for the often poor outcome after nerve injury in humans. Although some of the estimates vary, studies in rodents suggest that repair cells in distal nerve stumps maintain full capacity to support axonal regeneration for only about 2 months, with regeneration support dwindling by about 50% at 3–4 months in most studies (reviewed in Jessen and Mirsky 2019b). There is also direct evidence for a gradual reduction in the expression of molecular repair cell phenotypes. Levels of GDNF, BDNF, NGF, and NT3 all decline during prolonged denervation, and the same holds for repair cell markers such as P75NTR and Shh (Höke et al. 2002; Eggers et al. 2010; reviewed in Boyd and Gordon 2003; Höke and Brushart 2010). Ten weeks after injury, c-Jun protein is also reduced to about half of its peak levels, which is significant, since c-Jun is a global amplifier of the repair cell phenotype (see Sect. 7). Injury-induced Schwann cell elongation is also reversed with time. Between 4 and 10 weeks after injury repair, Schwann cells shorten by about 30%, and at 6 months they are only about half their maximum length (Gomez-Sanchez et al. 2017).

Additional to this phenotypic deterioration during long-term denervation, repair cell numbers gradually decline with time after injury. However, this only takes place after Schwann cell numbers have increased severalfold due to injury-induced proliferation, which means that Schwann cell numbers remain substantially higher than those in uninjured nerves for many months after injury. Since there is evidence that the increase in Schwann cell numbers after injury may be irrelevant for rate of axonal regeneration (Kim et al. 2000; Atanasoski et al. 2001, 2006; Yang et al. 2008), the relationship between reduction in cell numbers and decline in regeneration remains unclear, although continued loss of cells will ultimately hinder repair (reviewed in Jessen and Mirsky 2019b).

4 Repair Cell Failure and the Underlying Molecular Mechanisms

The repair cell deterioration that takes place during the long-term denervation caused by the slow rate of axon growth contributes significantly to poor recovery after nerve injury (reviewed in Höke 2006). Another factor preventing effective repair arises gradually during aging, since in many species, including rodents and humans, increased age is accompanied by decreased regeneration rates (reviewed in Verdú et al. 2000; Graciarena et al. 2014; Scheib and Höke 2013). Notably this appears not to be caused by age-related deterioration in neurons. Rather, the Schwann cells in aging animals show a subdued response to injury, expressing reduced levels of injury genes such as c-Jun (Painter et al. 2014; Wagstaff et al. 2017; reviewed in Painter 2017).

Thus, two apparently unrelated factors that both contribute to ineffective repair, chronic denervation and aging, involve a common component, namely, defective function of repair cells. Both types of regeneration failure have a major impact on the clinical outcome of nerve injuries. Therefore, in the future it will be important to define in more detail the molecular mechanisms responsible for repair cell decline during aging and long-term denervation.

At present, two transcription factors, c-Jun and STAT3, are implicated in upregulating the repair-supportive features of denervated Schwann cells and in maintaining these cells during long-term denervation. The function of c-Jun as a global amplifier of the repair cell phenotype, and role it plays in repair cell decline, both in aging and chronic denervation, is discussed in Sect. 7. STAT3 is activated by Tyr705 phosphorylation after nerve injury. This is sustained even during long-term denervation and serves a dual function as shown by experiments involving genetic inactivation of this protein selectively in Schwann cells (Benito et al. 2017). First, STAT3 is needed for autocrine Schwann cell survival signalling (Meier et al. 1999), and inactivation of STAT3 results in striking loss of repair cells from long-term denervated distal stumps. Second, STAT3 is required for maintaining repair cell properties. Inactivation of Schwann cell STAT3 leads to reduced expression of repair cell markers such as GDNF, BDNF, and Shh and abnormal morphology of repair cells and of the regeneration tracks (Bungner bands) that they form, a morphological

defect also seen in Schwann cells after c-Jun inactivation (Arthur-Farraj et al. 2012). The involvement of STAT3 in Schwann cells appear to be restricted to repair cells, since nerve development proceeds normally without Schwann cell STAT3 (Benito et al. 2017). Other signals that act primarily in repair cells are discussed in Sect. 7.

5 The Injured Nerve

The concept of the repair Schwann cell has been developed to describe Schwann cells occupying nerves distal to injury (Fig. 4). It is through the distal stump that axons navigate along the Bungner bands, relying on support from repair Schwann cells often for months before they reach their target tissues. The nature of the cells and regeneration events in the distal stump are similar irrespective of whether the injury involves axonal disruption with continuity of connective tissues (axonotmesis) or complete nerve interruption (neurotmesis). In the case of complete nerve interruption, however, an additional set of important events takes place in the injury region, where axons need to grow across the gap between the proximal and distal nerve stumps. This bridging tissue is formed by axon/Schwann cell columns (regeneration units) emerging from the proximal stump, and Schwann cells migrating from the distal stump, as well as connective tissue, macrophages, and blood vessels. The complex interactions between these cells are reviewed elsewhere (Cattin and Lloyd 2016).

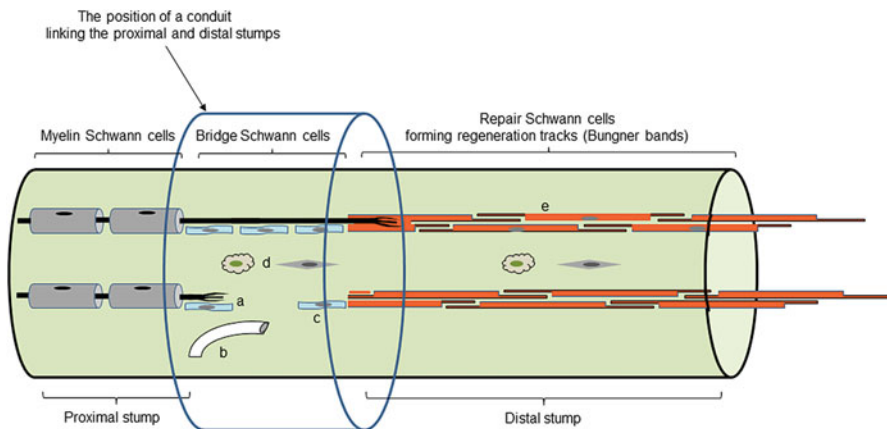


Fig. 4 Main cells and tissues of a regenerating nerve. Repair cells forming Bungner bands are dark orange (e). Schwann cells of the tissue bridge, some of which migrate out from the distal stump (c), are light blue, while other Schwann cells accompany regenerating axons forming regeneration units (a). The basal lamina that covers the Schwann cells in the proximal stump and the Bungner bands in the distal stump is not shown. The Schwann cells in the tissue bridge receive important signals from blood vessels (b), fibroblasts, and macrophages (d). The position of an inserted regeneration conduit is indicated (although the cellular events at this location depend on the nature of the conduit). (With permission from Jessen and Mirsky 2019b)

In injuries involving relatively little loss of nerve tissue, the gap occupied by a bridge is only microscopic since the proximal and distal stumps can be surgically attached. More extensive disruptive injuries necessitate the insertion of nerve autografts or, alternatively, regeneration conduits that provide artificial structures or tissues to support the growth of the axon/Schwann cell regeneration units across to the distal stump (Fig. 4) (Pabari et al. 2014). This approach is not needed in most compression or stretch injuries, since in these cases the supportive tissues of the nerve are typically intact, although axons are interrupted (axonotmesis). Clearly, connective and epithelial protective tissues are important factors in injured and regenerating nerves.

The protective tissue system of peripheral nerves consists of three components. First, the axon-Schwann cell units are directly embedded in the endoneurial connective tissue, namely, collagen fibers, other extracellular matrix (ECM) molecules, and associated fibroblasts. Second, the endoneurium containing axons and Schwann cells is encased in a cellular tube, the perineurium. In small nerves this may be a single layer of cells, but in large nerves the perineurial sheaths consist of many cell layers. Although generated from fibroblastic cells, this tissue exhibits key features of epithelia, since the perineurial cells are coated by a basal lamina and they are linked by tight junctions. The perineurium therefore provides a diffusion barrier against unwanted molecules, protects the nerve from invading cells, and helps maintain homeostasis of the endoneurial fluid. Third, outside the perineurium lies the collagen-rich connective tissue of the epineurium, which often contains adipocytes for further mechanical buffering.

Small nerves consist of a single perineurial tube containing the endoneurium and nerve fibers, a fascicle, while large nerves may contain 10–20 fascicles held together by the epineurium. The longitudinal organization of fascicles in multifascicular nerves is complex and incompletely understood (reviewed in Stewart 2003). Although some fascicles may run as uninterrupted cables along the nerve, fascicles also branch and split allowing axons from one fascicle to join another one. Such intermingling of fascicles is more prominent in proximal nerves. After disruptive nerve damage with minimal loss of nerve tissue, when surgical repair often involves the attachment of proximal and distal stumps directly, attention is paid to matching corresponding fascicles in the proximal and distal stump. Attempts at fascicular alignment are also thought to be important when nerves are repaired by insertion of autografts or conduits after extensive nerve damage. The anatomy of fascicles, and how it relates to the grouping of axons with similar function or common target tissues, is clinically relevant and one of the factors that determine the success of repair.

6 The Nerve Protective Tissues Are Organized by Developing Schwann Cells

All three protective systems of peripheral nerves, the endo-, peri-, and epineurium, are organized by Schwann cells in early developing nerves (Parmentier et al. 1999; Umehara et al. 2000; Sharghi-Namini et al. 2006; Fig. 5).

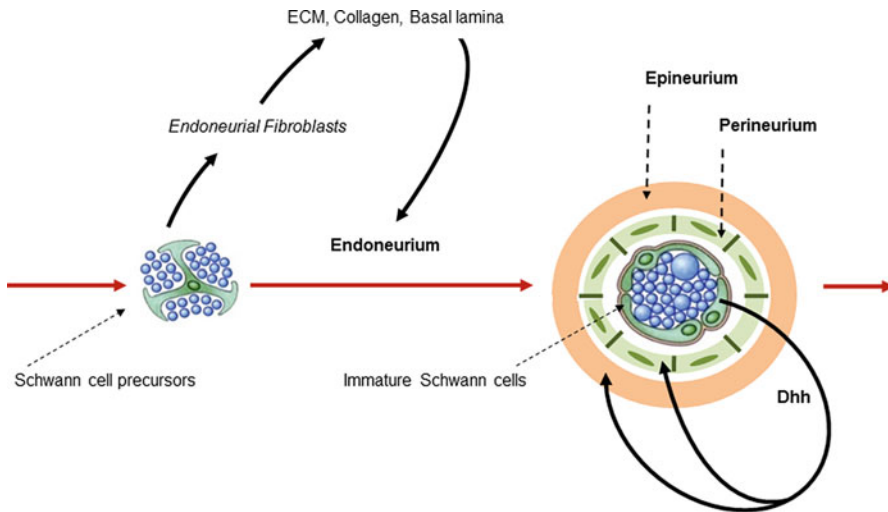


Fig. 5 Developing Schwann cells control the formation of the connective tissues of peripheral nerves: endoneurium, perineurium, and epineurium. Schwann cell precursors give rise to endoneurial fibroblasts, cells specialized for generating extracellular matrix and connective tissue. Developing Schwann cells express Desert hedgehog (Dhh), a protein that is essential for the development of the perineurium and epineurium, structures that are not fully formed until postnatal stages later than that shown here

In early nerves, the first defined stage of Schwann cell lineage is the Schwann cell precursor (SCP), which is generated from the neural crest (Fig. 1) (reviewed in Jessen and Mirsky 2005, 2019a; Monk et al. 2015; Jessen et al. 2015b). SCPs like other glial cells are tightly associated with axons, and they express glial/Schwann cell-associated factors, including *MPZ*, *DHH* (desert hedgehog), *Fabp7* (BFABP), and Connexin 29 (Li et al. 2007). These cells show one interesting similarity with developing glial cells in the CNS, the radial glia, namely, developmental multipotency (reviewed in Malatesta and Götz 2013; Jessen and Mirsky 2019a). In the case of SCP, these cells generate endoneurial fibroblasts (Joseph et al. 2004 see below) and contribute to other cell groups including melanocytes and autonomic neurons, in addition to their main fate, which is the generation of immature Schwann cells (reviewed in Jessen and Mirsky 2019a).

SCPs convert to immature Schwann cells at E15–E17 in the rat (E13–E15 in mouse). This coincides with histological transformation of developing nerves, involving the opening up of endoneurial connective tissue spaces containing extracellular matrix and blood vessels and the formation of the Schwann cell basal lamina (Wanner et al. 2006; reviewed in Jessen and Mirsky 2019a; Jessen et al. 2015b). Around birth in rodents, immature Schwann cells diversify, with cells that randomly associate with larger axons forming myelin subsequent to radial sorting, while cells associated with smaller axons becoming Remak cells, typically forming multi-axonal Remak fibers (Salzer 2015; Harty and Monk 2017).

The development of human Schwann cells appears to follow a similar sequence during embryonic and early postnatal development (Gamble 1966).

In SCPs, apoptotic death is the default pathway, and they therefore depend on continuous axonal signals, in particular neuregulin1, for survival (Jessen et al. 1994; Dong et al. 1995). Immature Schwann cells, in contrast, survive without touching axons; their survival is promoted by autocrine survival loops that are not present in the precursors (Meier et al. 1999). This switch to axon-independent survival is of central importance in injured nerves. The death of damaged axons results, in the short and medium term, in a mild and transient wave of Schwann cell death only, with the bulk of the denervated Schwann cells surviving to support axonal regeneration.

The developing Schwann cells described above work through two distinct mechanisms to organize the protective sheaths of peripheral nerves (Fig. 5). First, SCP and immature Schwann cells in embryonic nerves secrete the signalling protein Desert hedgehog (Dhh), which is essential for the generation of both the cellular perineurial tube and of the epineurial connective tissue. In Dhh knockout mice, the wall of the perineurial tube is disorganized and has a reduced number of cells, which are covered by a patchy basal lamina and often invade the endoneurial space. The tight junctions between the perineurial cells are structurally abnormal, and the perineurium no longer acts as a diffusion barrier (Parmantier et al. 1999). The collagenous epineurium is substantially reduced in thickness and often almost absent (Parmantier et al. 1999). Second, as mentioned above, in addition to generating Schwann cells, SCPs give rise to the fibroblasts of the endoneurium. These cells, in turn, are instrumental in forming the endoneurial connective tissue.

Increasing attention is now being paid to the way mechanical forces that arise from the interactions of Schwann cells with connective tissue or axons are transduced and how these signals affect Schwann cell behavior. One example is the different length of myelin internodes in adult uninjured and regenerated nerves. In adults, where mechanical stretching associated with developmental increase in limb size is absent, the lengths of myelin sheaths formed after injury and regeneration are much shorter than the myelin sheaths formed during development and found in uninjured adult nerves (Gomez-Sanchez et al. 2017). It has been suggested that the mechanical stretching of the nerve that occurs as the animal grows during development contributes to this difference and that stretching promotes Schwann cell proliferation, radial sorting, myelination, and axon elongation (Belin et al. 2017; Tricaud 2018). The YAP/TAZ pathway has been implicated in the transduction of mechanical forces in Schwann cells. This pathway, part of the Hippo signalling pathway, controls Schwann cell proliferation and is activated by mechanical stimulation. Severe radial sorting and myelination defects are seen in mice with Schwann cell-specific knockout of both YAP and TAZ together (Poitelon et al. 2016; Deng et al. 2017; Grove et al. 2017). In addition, in Merlin-null mice, internodal length of adult myelinated fibers is short, a defect that is corrected in YAP null Merlin-null nerves, again emphasizing the importance of YAP in controlling normal internodal length. It could therefore be advantageous to use mechanical transduction strategies

to promote optimal axon growth and myelination after nerve injury not only in the injured distal stump but also in nerve regeneration conduits.

7 The Schwann Cell Injury Response Is Controlled by Special Signalling Mechanisms

Multiple intracellular pathways and transcription factors contribute to the reprogramming of Schwann cells after injury. Some of these signals are also implicated in Schwann cell development. But recently it has become clear that repair cell generation is also controlled by dedicated signals that have relatively little or no function in development. This finding is in line with the notion that Schwann cells in injured nerves represent a specialized repair phenotype that is distinct from other cells in the lineage.

Among the signalling systems that have defined roles both in development and during injury and/or regeneration are ERK1/2, histone deacetylase (HDAC)2, and the transcriptional regulators Notch and Zeb2 (reviewed in Boerboom et al. 2017; Quintes and Brinkmann 2017; Jessen and Arthur-Farraj 2018).

The signals that are important in repair cells, but not in Schwann cell development, include the transcription factors c-Jun and STAT3 (discussed in an earlier section), merlin, cyclin D1, and epigenetic mechanisms involving H3K27 H3K4.

c-Jun is a prominent example of a signal of this kind (Arthur-Farraj et al. 2012; reviewed in Jessen and Mirsky 2016, 2019b; Jessen and Arthur-Farraj 2018). Mice with genetic inactivation of c-Jun selectively in Schwann cells show no obvious developmental defects, and adult nerves are normal. But the repair Schwann cells generated after injury are dysfunctional. This results in increased death of injured neurons, reduced axonal regeneration, and severely compromised functional recovery. Some 4000–6000 genes change expression in nerves distal to injury. This includes c-Jun, which is elevated 80- to 100-fold at the protein level. In c-Jun knockout mice, a discrete number of these genes, less than 200, are dysregulated. This includes trophic factors such as GDNF and BDNF as well as myelin genes, explaining the generation of faulty repair cells in the absence of c-Jun. Importantly, the converse is also true, since enforced elevation of c-Jun can be employed to generate enhanced repair cells. In Schwann cell implants, where regeneration in wild-type control mice is suboptimal, enforced c-Jun expression elevates trophic factor expression and promotes axonal regeneration (Huang et al. 2019). The same beneficial effect is seen in other situations where regeneration is ineffective, such as in aging animals and after chronic denervation. In both of these situations, experimentally enhanced expression of c-Jun specifically in repair cells promotes axonal regeneration (Wagstaff et al. 2017). These findings establish c-Jun as a global amplifier of the repair Schwann cell phenotype and point to the c-Jun pathway as a potential target for therapeutic interventions to improve nerve repair (reviewed in Jessen and Mirsky 2016, 2019b; Jessen and Arthur-Farraj 2018).

Merlin is a tumor suppressor protein that has only a minor role in Schwann cell development but is crucial in repair cells (Mindos et al. 2017; Truong et al. 2018). If

Merlin is selectively inactivated in Schwann cells, axonal regeneration after injury is strongly suppressed, re-myelination of those axons that regenerate is impaired, and the connective tissue compartment of the injured nerve is enlarged. The molecular mechanisms underlying these effects involve overexpression of the Hippo pathway effector YAP, and the defects in Merlin-null animals are largely corrected by specific knockout of YAP. Other aspects of the underlying signalling mechanisms are unclear, although in Merlin knockout Schwann cells, c-Jun levels are lower, and changes in ERK activation occur (Mindos et al. 2017).

Epigenetic mechanisms with a role selectively in the Schwann cell injury response have also been described, involving H3K27 demethylation, H3K27 acetylation, and H3K4 methylation, events that collectively facilitate the downregulation of myelin genes and upregulation of injury genes in repair cells (Hung et al. 2015; reviewed in Ma and Svaren 2018).

The molecular control of proliferation also differs between immature Schwann cells during development and repair cells after injury, since cyclin D1 controls proliferation of repair cells but not that of immature Schwann cells (Kim et al. 2000; Atanasoski et al. 2001, 2006; Yang et al. 2008).

Further, some of the molecules with a role in both development and injury function differently in the two situations. Thus after injury, repair cells activate an autocrine neuregulin signalling loop to promote re-myelination of regenerated axons, although this mechanism is not present during developmental myelination by immature Schwann cells (Fricker et al. 2013; Stassart et al. 2013). Surprisingly also, although neuregulin facilitates myelination in injured nerves and regulates the timing of myelin formation, this factor is not essential for re-myelination. In contrast, neuregulin is required for developmental myelination. Lastly, ERK1/2 activation promotes myelin thickness during development, but does not affect the thickness of myelin around regenerated axons (Cervellini et al. 2018; Ishii et al. 2013).

8 Conclusions

Nerve repair is heavily reliant on the injury-induced reprogramming of Schwann cells to a repair phenotype specialized for supporting injured neurons and axonal regeneration. The signalling mechanisms that control these repair Schwann cells include dedicated signals that have relatively little or no function in development. In the future, it will be important to learn more about the distinctive molecular pathways that control the performance and long-term maintenance of repair Schwann cells, since this will facilitate the search for ways to manipulate these mechanisms for promoting regeneration.

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References

- Allodi I, Udina E, Navarro X (2012) Specificity of peripheral nerve regeneration: interactions at the axon level. *Prog Neurobiol* 98:16–37
- Armstrong SJ, Wiberg M, Terenghi G, Kingham PJ (2007) ECM molecules mediate both Schwann cell proliferation and activation to enhance neurite outgrowth. *Tissue Eng* 13:2863–2870
- Arthur-Farraj PJ, Latouche M, Wilton DK, Quintes S, Chabrol E, Banerjee A, Woodhoo A, Jenkins B, Rahman M, Turmaine M, Wicher GK, Mitter R, Greensmith L, Behrens A, Raivich G, Mirsky R, Jessen KR (2012) c-Jun reprograms Schwann cells of injured nerves to generate a repair cell essential for regeneration. *Neuron* 75:633–647
- Arthur-Farraj PJ, Morgan CC, Adamowicz M, Gomez-Sanchez JA, Fazal SV, Beucher A, Razzaghi B, Mirsky R, Jessen KR, Aitman TJ (2017) Changes in the coding and non-coding transcriptome and DNA methylome that define the Schwann cell repair phenotype after nerve injury. *Cell Rep* 20:2719–2734
- Atanasoski S, Shumas S, Dickson C, Scherer SS, Suter U (2001) Differential cyclin D1 requirements of proliferating Schwann cells during development and after injury. *Mol Cell Neurosci* 18:581–592
- Atanasoski S, Scherer SS, Sirkowski E, Leone D, Garratt AN, Birchmeier C, Suter U (2006) ErbB2 signaling in Schwann cells is mostly dispensable for maintenance of myelinated peripheral nerves and proliferation of adult Schwann cells after injury. *J Neurosci* 26:2124–2131
- Belin S, Zuloaga KL, Poitelon Y (2017) Influence of mechanical stimuli on Schwann cell biology. *Front Cell Neurosci* 11:347
- Benito C, Davis CM, Gomez-Sanchez JA, Turmaine M, Meijer D, Poli V, Mirsky R, Jessen KR (2017) STAT3 controls the long-term survival and phenotype of repair Schwann cells during nerve regeneration. *J Neurosci* 37:4255–4269
- Blesch A, Lu P, Tsukada S, Alto LT, Roet K, Coppola G, Geschwind D, Tuszynski MH (2012) Conditioning lesions before or after spinal cord injury recruit broad genetic mechanisms that sustain axonal regeneration: superiority to cAMP-mediated effects. *Exp Neurol* 235:162–173
- Boerboom A, Dion V, Chariot A, Franzen R (2017) Molecular mechanisms involved in Schwann cell plasticity. *Front Mol Neurosci* 10:38
- Boyd JG, Gordon T (2003) Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. *Mol Neurobiol* 27:277–324
- Bradke F, Fawcett JW, Spira ME (2012) Assembly of a new growth cone after axotomy: the precursor to axon regeneration. *Nat Rev Neurosci* 13:183–193
- Campana WM (2007) Schwann cells: activated peripheral glia and their role in neuropathic pain. *Brain Behav Immun* 21:522–527
- Castelnovo LF, Bonalume V, Melfi S, Ballabio M, Colleoni D, Magnaghi V (2017) Schwann cell development, maturation and regeneration: a focus on classic and emerging intracellular signaling pathways. *Neural Regen Res* 12:1013–1023
- Cattin AL, Lloyd AC (2016) The multicellular complexity of peripheral nerve regeneration. *Curr Opin Neurobiol* 39:38–46
- Cervellini I, Galino J, Zhu N, Allen S, Birchmeier C, Bennett DL (2018) Sustained MAPK/ERK activation in adult Schwann cells impairs nerve repair. *J Neurosci* 38:679–690
- Chen ZL, Yu WM, Strickland S (2007) Peripheral regeneration. *Annu Rev Neurosci* 30:209–233
- Chera S, Baronnier D, Ghila L, Cigliola V, Jensen JN, Gu G, Furuyama F, Thorel F, Gribble FM, Reimann F, Herrera PL (2014) Diabetes recovery by age-dependent conversion of pancreatic δ -cells into insulin producers. *Nature* 514:503–507
- Clements MP, Byrne E, Camarillo Guerrero LF, Cattin AL, Zakka L, Ashraf A, Burden JJ, Khadayate S, Lloyd AC, Marguerat S, Parrinello S (2017) The wound microenvironment reprograms Schwann cells to invasive mesenchymal-like cells to drive peripheral nerve regeneration. *Neuron* 96:98–114
- Deng Y, Wu LMN, Bai S, Zhao C, Wang H, Wang J, Xu L, Sakabe M, Zhou W, Xin M, Lu QR (2017) A reciprocal regulatory loop between TAZ/YAP and G-protein G α s regulates Schwann cell proliferation and myelination. *Nat Commun* 8:15161

- Dimou L, Gallo V (2015) NG2-glia and their functions in the central nervous system. *Glia* 63:1429–1451
- Dong Z, Brennan A, Liu N, Yarden Y, Lefkowitz G, Mirsky R, Jessen KR (1995) Neu differentiation factor is a neuron-glia signal and regulates survival, proliferation, and maturation of rat Schwann cell precursors. *Neuron* 15:585–596
- Doron-Mandel E, Fainzilber M, Terenzio M (2015) Growth control mechanisms in neuronal regeneration. *FEBS Lett* 589:1669–1677
- Dumont NA, Bentzinger CF, Sincennes MC, Rudnicki MA (2015) Satellite cells and skeletal muscle regeneration. *Compr Physiol* 5:1027–1059
- Eggers R, Tannemaat MR, Ehler EM, Verhaagen J (2010) A spatio-temporal analysis of motoneuron survival, axonal regeneration and neurotrophic factor expression after lumbar ventral root avulsion and implantation. *Exp Neurol* 223:207–220
- Fawcett JW, Verhaagen J (2018) Intrinsic determinants of axon regeneration. *Dev Neurobiol* 78:890–897
- Fricker FR, Antunes-Martins A, Galino J, Paramsothy R, La Russa F, Perkins J, Goldberg R, Brelstaff J, Zhu N, McMahon SB, Orenco C, Garratt AN, Birchmeier C, Bennett DL (2013) Axonal neuregulin 1 is a rate limiting but not essential factor for nerve remyelination. *Brain* 136:2279–2297
- Fu SY, Gordon T (1997) The cellular and molecular basis of peripheral nerve regeneration. *Mol Neurobiol* 14:67–116
- Gamble HJ (1966) Further electron microscope studies of human foetal peripheral nerves. *J Anat* 100:487–502
- Gomez-Sanchez JA, Pilch KS, van der Lans M, Fazal SV, Benito C, Wagstaff LJ, Mirsky R, Jessen KR (2017) After nerve injury, lineage tracing shows that myelin and Remak Schwann cells elongate extensively and branch to form repair Schwann cells, which shorten radically on remyelination. *J Neurosci* 37:9086–9099
- Graciarena M, Dambly-Chaudière C, Ghysen A (2014) Dynamics of axonal regeneration in adult and aging zebrafish reveal the promoting effect of a first lesion. *Proc Natl Acad Sci USA* 111:1610–1615
- Graf T, Enver T (2009) Forcing cells to change lineages. *Nature* 462:587–594
- Grove M, Kim H, Santerre M, Krupka AJ, Han SB, Zhai J, Cho JY, Park R, Harris M, Kim S, Sawaya BE, Kang SH, Barbe MF, Cho SH, Lemay MA, Son YJ (2017) YAP/TAZ initiate and maintain Schwann cell myelination. *Elife* 6. pii: e20982
- Harty BL, Monk KR (2017) Unwrapping the unappreciated: recent progress in Remak Schwann cell biology. *Curr Opin Neurobiol* 47:131–137
- Höke A (2006) Mechanisms of disease: what factors limit the success of peripheral nerve regeneration in humans? *Nat Clin Pract Neurol* 2:448–454
- Höke A, Brushart T (2010) Challenges and opportunities for regeneration in the peripheral nervous system. *Exp Neurol* 223:1–4
- Höke A, Gordon T, Zochodne DW, Sulaiman OA (2002) A decline in glial cell-line-derived neurotrophic factor expression is associated with impaired regeneration after long-term Schwann cell denervation. *Exp Neurol* 173:77–85
- Huang L, Xia B, Shi X, Gao J, Yang Y, Xu F, Qi F, Liang C, Huang J, Luo Z (2019) Time-restricted release of multiple neurotrophic factors promotes axonal regeneration and functional recovery after peripheral nerve injury. *FASEB J* 33:8600–8613
- Hung HA, Sun G, Keles S, Svaren J (2015) Dynamic regulation of Schwann cell enhancers after peripheral nerve injury. *J Biol Chem* 290:6937–6950
- Ishii A, Furusho M, Bansal R (2013) Sustained activation of ERK1/2 MAPK in oligodendrocytes and Schwann cells enhances myelin growth and stimulates oligodendrocyte progenitor expansion. *J Neurosci* 33:175–186
- Jessen KR, Arthur-Farraj P (2018) Repair Schwann cell update: adaptive reprogramming, EMT, and stemness in regenerating nerves. *Glia* 67:421–437
- Jessen KR, Mirsky R (2005) The origin and development of glial cells in peripheral nerves. *Nat Rev Neurosci* 6:671–682

- Jessen KR, Mirsky R (2016) The repair Schwann cell and its function in regenerating nerves. *J Physiol* 594:3521–3531
- Jessen KR, Mirsky R (2019a) Schwann cell precursors: multipotent glial cells in embryonic nerves. *Front Mol Neurosci* 12:69
- Jessen KR, Mirsky R (2019b) The success and failure of the Schwann cell response to injury. *Front Cell Neurosci* 13:33
- Jessen KR, Brennan A, Morgan L, Mirsky R, Kent A, Hashimoto Y, Gavrilovic J (1994) The Schwann cell precursor and its fate: a study of cell death and differentiation during gliogenesis in rat embryonic nerves. *Neuron* 12:509–527
- Jessen KR, Mirsky R, Arthur-Farraj P (2015a) The role of cell plasticity in tissue repair: adaptive cellular reprogramming. *Dev Cell* 34:613–620
- Jessen KR, Mirsky R, Lloyd AC (2015b) Schwann cells: development and role in nerve repair. *Cold Spring Harb Perspect Biol* 7(7). <https://doi.org/10.1101/cshperspect.a020487>
- Joseph NM, Mukouyama YS, Mosher JT, Jaegle M, Crone SA, Dormand EL, Lee KF, Meijer D, Anderson DJ, Morrison SJ (2004) Neural crest stem cells undergo multilineage differentiation in developing peripheral nerves to generate endoneurial fibroblasts in addition to Schwann cells. *Development* 131:5599–5612
- Kim HA, Pomeroy SL, Whoriskey W, Pawlitzky I, Benowitz LI, Sicinski P, Stiles CD, Roberts TM (2000) A developmentally regulated switch directs regenerative growth of Schwann cells through cyclin D1. *Neuron* 26:405–416
- Li J, Habbes HW, Eiberger J, Willecke K, Dermietzel R, Meier C (2007) Analysis of connexin expression during mouse Schwann cell development identifies connexin 29 as a novel marker for the transition of neural crest to precursor cells. *Glia* 55:93–103
- Ma KH, Svaren J (2018) Epigenetic control of Schwann cells. *Neuroscientist* 24:627–638
- Malatesta P, Götz M (2013) Radial glia – from boring cables to stem cell stars. *Development* 140:483–486
- Meier C, Parmantier E, Brennan A, Mirsky R, Jessen KR (1999) Developing Schwann cells acquire the ability to survive without axons by establishing an autocrine circuit involving insulin-like growth factor, neurotrophin-3, and platelet-derived growth factor-BB. *J Neurosci* 19:3847–3859
- Mindos T, Dun XP, North K, Doddrell RD, Schulz A, Edwards P, Russell J, Gray B, Roberts SL, Shivane A, Mortimer G, Pirie M, Zhang N, Pan D, Morrison H, Parkinson DB (2017) Merlin controls the repair capacity of Schwann cells after injury by regulating Hippo/YAP activity. *J Cell Biol* 216:495–510
- Monk KR, Feltri ML, Taveggia C (2015) New insights on Schwann cell development. *Glia* 63:1376–1393
- Nieto MA, Huang RY, Jackson RA, Thiery JP (2016) EMT: 2016. *Cell* 166:21–45
- Pabari A, Lloyd-Hughes H, Seifalian AM, Mosahebi A (2014) Nerve conduits for peripheral nerve surgery. *Plast Reconstr Surg* 133:1420–1430
- Painter MW (2017) Aging Schwann cells: mechanisms, implications, future directions. *Curr Opin Neurobiol* 47:203–208
- Painter MW, Brosius Lutz A, Cheng YC, Latremoliere A, Duong K, Miller CM, Posada S, Cobos EJ, Zhang AX, Wagers AJ, Havton LA, Barres B, Omura T, Woolf CJ (2014) Diminished Schwann cell repair responses underlie age-associated impaired axonal regeneration. *Neuron* 83:331–343
- Parmantier E, Lynn B, Lawson D, Turmaine M, Namini SS, Chakrabarti L, McMahon AP, Jessen KR, Mirsky R (1999) Schwann cell-derived desert hedgehog controls the development of peripheral nerve sheaths. *Neuron* 23:713–724
- Poitelon Y, Lopez-Anido C, Catignas K, Berti C, Palmisano M, Williamson C, Ameroso D, Abiko K, Hwang Y, Gregorieff A, Wrana JL, Asmani M, Zhao R, Sim FJ, Wrabetz L, Svaren J, Feltri ML (2016) YAP and TAZ control peripheral myelination and the expression of laminin receptors in Schwann cells. *Nat Neurosci* 19:879–887
- Quintes S, Brinkmann BG (2017) Transcriptional inhibition in Schwann cell development and nerve regeneration. *Neural Regen Res* 12:1241–1246

- Salzer JL (2015) Schwann cell myelination. *Cold Spring Harb Perspect Biol* 7(8):a020529
- Scheib J, Höke A (2013) Advances in peripheral nerve regeneration. *Nat Rev Neurol* 9:668–676
- Sharghi-Namini S, Turmaine M, Meier C, Sahni V, Umehara F, Jessen KR, Mirsky R (2006) The structural and functional integrity of peripheral nerves depends on the glial-derived signal desert hedgehog. *J Neurosci* 26:6364–6376
- Shen CN, Burke ZD, Tosh D (2004) Transdifferentiation, metaplasia and tissue regeneration. *Organogenesis* 1:36–44
- Sisakhtnezhad S, Matin MM (2012) Transdifferentiation: a cell and molecular reprogramming process. *Cell Tissue Res* 348:379–396
- Skrypek N, Goossens S, De Smedt E, Vandamme N, Berx G (2017) Epithelial-to-mesenchymal transition: epigenetic reprogramming driving cellular plasticity. *Trends Genet* 33:943–959
- Stassart RM, Fledrich R, Velanac V, Brinkmann BG, Schwab MH, Meijer D, Sereda MW, Nave KA (2013) A role for Schwann cell-derived neuregulin-1 in remyelination. *Nat Neurosci* 16:48–54
- Stewart JD (2003) Peripheral nerve fascicles: anatomy and clinical relevance. *Muscle Nerve* 28(5):525–541
- Thorel F, Népote V, Avril I, Kohno K, Desgraz R, Chera S, Herrera PL (2010) Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. *Nature* 464:1149–1154
- Tricaud N (2018) Myelinating Schwann cell polarity and mechanically-driven myelin sheath elongation. *Front Cell Neurosci* 11:414
- Truong K, Ahmad I, Jason Clark J, Seline A, Bertroche T, Mostaert B, Van Daele DJ, Hansen MR (2018) Nf2 mutation in Schwann cells delays functional neural recovery following injury. *Neuroscience* 374:205–213
- Tsonis PA, Madhavan M, Tancous EE, Del Rio-Tsonis K (2004) A newt's eye view of lens regeneration. *Int J Dev Biol* 48:975–980
- Umehara F, Tate G, Itoh K, Yamaguchi N, Douchi T, Mitsuya T, Osame M (2000) A novel mutation of desert hedgehog in a patient with 46, XY partial gonadal dysgenesis accompanied by minifascicular neuropathy. *Am J Hum Genet* 67:1302–1305
- Vargas ME, Barres BA (2007) Why is Wallerian degeneration in the CNS so slow? *Annu Rev Neurosci* 30:153–179
- Verdú E, Ceballos D, Vilches JJ, Navarro XJ (2000) Influence of aging on peripheral nerve function and regeneration. *J Peripher Nerv Syst* 5:191–208
- Wagstaff L, Gomez-Sanchez J, Mirsky R, Jessen KR (2017) The relationship between Schwann cell c-Jun and regeneration failures due to aging and long-term injury. *Glia* 65:E532
- Wanner IB, Mahoney J, Jessen KR, Wood PM, Bates M, Bunge MB (2006) Invariant mantling of growth cones by Schwann cell precursors characterize growing peripheral nerve fronts. *Glia* 54:424–438
- Webber C, Zochodne D (2010) The nerve regenerative microenvironment: early behavior and partnership of axons and Schwann cells. *Exp Neurol* 223:51–59
- Yang DP, Zhang DP, Mak KS, Bonder DE, Pomeroy SL, Kim HA (2008) Schwann cell proliferation during Wallerian degeneration is not necessary for regeneration and remyelination of the peripheral nerves: axon-dependent removal of newly generated Schwann cells by apoptosis. *Mol Cell Neurosci* 38:80–88

Therapeutic Cells and Stem Cells for Nerve Regeneration

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Abstract

Although there is a considerable regenerative potential in the peripheral nervous system, recovery after traumatic nerve injuries, especially in the case of severe lesions, is incomplete. Despite the recent improvements in microsurgical techniques and the development of new nerve conduits that have widened the availability of tools to achieve more successful reinnervation of the peripheral targets, the clinical outcome is often still not satisfactory. The availability of autologous Schwann cells as donor cell types is very limited and their use from allogeneic sources raises a number of questions. Therefore the use of the regeneration-promoting effect of stem cells in peripheral nerve regeneration following injuries appears to be crucial. Multi- or pluripotent stem cells with a self-renewing capacity may be taken from several sources for preclinical applications and their use proved to be very promising to induce improved morphological and functional recovery in the injured peripheral nervous system.

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The future opportunities along with the pros and cons of stem cell-based therapies to improve peripheral nerve regeneration are discussed in this chapter.

1 Introduction

Given that Schwann cells play a key role in regeneration following peripheral nerve injury, Schwann cells have been transplanted that have improved the rate of functional regeneration in various animal models (Rodrigues et al. 2012). However, Schwann cell transplantation raises a number of problems, such as limited extensions in vitro, sourcing of allogeneic Schwann cells (Fairbairn et al. 2015), etc. Therefore, there is great interest in alternative cell sources, such as adipose-derived stem cells (ADSCs), bone marrow stromal cells (BMSCs), umbilical cord stem cells, skin-derived precursor cells, and induced pluripotent stem cells (iPSCs), which have excellent self-renewing capacity and a great potential for differentiation (Xu et al. 2008; Polak and Bishop 2006; Sowa et al. 2016; Caseiro et al. 2016; Grochmal et al. 2014; Liu et al. 2012). Identifying and selecting the most appropriate stem cell sources to enhance peripheral nerve regeneration remains a significant challenge. The benefits of stem cell therapy are numerous and individual stem cell sources can have many advantages, but also disadvantages (Fairbairn et al. 2015; Bhangra et al. 2016). Ideally, the cells should be easily isolated from the patient to allow autologous therapy and thus avoid immunosuppressive treatments, although allogeneic sources may also provide a good alternative if a deleterious immunological response is avoided. Another important property is that of transplanted cells is that they have similar phenotypic characteristics as Schwann cells and produce trophic factors that promote peripheral nerve regeneration (Bhangra et al. 2016).

Stem cells are potential candidates for use in peripheral nerve regeneration. In the following chapter, we focus on a variety of stem cells that have already been evaluated in animal models of peripheral nerve injury.

2 Bone Marrow Stromal Cells for Repair of Peripheral Nerve Lesion

BMSCs also known as human skeletal stem cells are located in a perivascular niche in close association with endothelial cells and pericytes within the bone marrow stroma and have a strong self-renewal capacity and multipotent differentiation in vitro into mesenchymal lineages (Abdallah and Kassem 2008; Crisan et al. 2008; Bianco and Robey 2015). Traditionally, BMSCs have been isolated based on expression of specific surface CD markers such as CD29, CD44, CD73, CD105, CD106, CD146, and CD166 (Abdallah and Kassem 2008). BMSCs are multipotential stem cells that, under specific conditions, are able to differentiate into osteoblasts, chondroblasts, and adipocytes (Abdallah and Kassem 2008). However, they

have the potential to differentiate into neurons or astrocytes *in vitro* and *in vivo*, but can also be persuaded to adopt a Schwann cell phenotype (Woodbury et al. 2000; Deng et al. 2001; Eglitis and Mezey 1997; Dezawa et al. 2001).

The cultured fibroblast-like populations of BMSCs synthesize and secrete neurotrophic factors and extracellular matrix components (nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial-cell-line-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF), collagen I, collagen IV, fibronectin, and laminin (Chen et al. 2007). Chen has also shown that fibroblast-like BMSC implantation into the injured rat spinal cord is able to promote regeneration and induce functional recovery (Chen et al. 2007). Furthermore, regenerative process after BMSC transplantation is accompanied by elevated expression of P0, PMP22, NGF, BDNF, GDNF, and CNTF in the regenerated tissue (Chen et al. 2007) providing further evidence that BMSCs have beneficial effect on nerve regeneration mainly on a humoral basis. In another study, the effect of BMSCs on functional recovery after peripheral nerve lesion was compared with that of Schwann cells tested with electrophysiology and functional assessments (Zarbakhsh et al. 2012). In this latter study, a 12-mm-long Schwann cells- or BMSCs-filled silicone tube was interposed into the rat sciatic nerve gap and BMSCs were able to generate equivalent recovery as Schwann cells 12 weeks after grafting.

BMSCs have also been studied in larger animal models, including rabbits and nonhuman primates (Wang et al. 2015; Hu et al. 2007). Wang used a rabbit model of 10 mm sciatic nerve defects where BMSCs were applied with a polylactic glycolic acid conduit and extracellular matrix gel construct. In this scenario, the sciatic nerves of the animals were completely regenerated and showed well myelination and regularly arranged nerve fibers. In a primate large animal model, chemically extracted acellular allogenic nerve grafts were implanted with autologous BMSCs to repair a 40 mm gap in the ulnar nerve of rhesus monkeys (Hu et al. 2007). At 6 months after grafting, the electrophysiological data of BMSCs-treated animals showed similar functional recovery compared with autologous nerve grafts.

In clinical practice, autologous nerve transplants represent the gold standard. However, autologous nerve transplantation has limitations, especially when long nerve grafts or different diameters of grafted nerves are required to repair peripheral nerve gap. BMSCs have not been used in the clinic to repair peripheral nerve injury so far, but based on successful animal experiments, they may be a prime candidate for translational study.

3 Induced Pluripotent Stem Cells and Their Derivatives

iPSCs have the capacity to differentiate all three germ layers and thus have been used for the development of new drugs, understanding the mechanisms of diseases, and for regenerative medicine with or without gene repair (Narazaki et al. 2008; Hanna et al. 2007; Xu et al. 2009; Yoshida and Yamanaka 2010). The first reprogramming

of cultured fibroblasts was achieved by ectopic expression of four transcription factors (Oct3/4, Sox2, c-Myc, and Klf4) (Takahashi et al. 2007; Takahashi and Yamanaka 2006). Since then a variety of methods have been developed for reprogramming somatic cells to iPSCs (Yu et al. 2007; Stadtfeld et al. 2008a, b; Maherali et al. 2008; Somers et al. 2010; Zhou et al. 2009; Zhou and Freed 2009; Si-Tayeb et al. 2010; Fusaki et al. 2009; Kim et al. 2009). Reprogramming results in a wide range of colonies appearing morphologically similar to embryonic stem cells; however, only a subset of these have comparable molecular and functional features. iPSCs show pluripotent stem cells properties including the differentiation potentials, the tumorigenic activities (teratoma-forming potentials), and the high proliferative capacity. On the other hand, iPSCs represent a greater risk to turn tumorigenic than embryonic stem cells, because foreign genes are introduced into the chromosome. Furthermore, the efficacy of transplanted iPSCs largely depends on the origin of somatic cells (Yamanaka 2020; Okita et al. 2007).

In the last decade several authors have shown that iPSCs may open a new avenue in the nervous system regeneration field (Wang et al. 2011; Tsuji et al. 2010). Few studies have provided evidence that iPSCs have the ability to differentiate into Schwann cells (Liu et al. 2012; Ma et al. 2015). The iPSC-derived Schwann cells expressed S100 β , P75^{NTR}, ERBB3, as well as the myelin markers PMP22 and MBP (Liu et al. 2012).

Wang et al. (2011) investigated the therapeutic potential of iPSC-derived neural crest stem cells for peripheral nerve injury (Wang et al. 2011). The human iPSC derivatives were applied with nanofibrous tubular scaffolds and used to bridge rat sciatic nerve gap lesions. Both morphological and electrophysiological analysis demonstrated that iPSC-derived neural crest stem cells seeded in nerve conduits resulted in an accelerated regeneration of sciatic nerves at 1 month after injury. Furthermore, the grafted cells expressed Schwann cell-specific markers (S100 β) and contributed to the myelination of axons.

iPSCs have the ability to differentiate into neural precursor cell aggregates, so-called neurospheres (Tsuji et al. 2010). Primary neurospheres had neurogenic differentiation potential but displayed no therapeutic effects on spinal cord injury, whereas secondary neurospheres were able to promote remyelination and axonal growth leading to improved function (Tsuji et al. 2010). The gliogenic secondary neurospheres had the ability to differentiate into Schwann cells and the bioabsorbable nerve conduits created by 3D-culture of iPS cell-derived secondary neurospheres induced peripheral nerve regeneration and motor function recovery (Uemura et al. 2012). However, it is not well clarified in this study through which mechanism the iPS-derived neurospheres promote the regeneration of peripheral nerves. Another interesting study has reported on the regenerative effect of iPSC-derived neurons grafted into the distal stump of transected sciatic nerves (Pepper et al. 2017). The iPSC-derived motoneurons extended neurites and formed neuromuscular junctions on denervated muscle fibers thus reducing denervation-induced muscular atrophy.

Although application of autologous iPSC-derived Schwann cells is not yet possible, the positive results of rodents experiments give cause for hope in this area. A number of questions need to be answered before iPSC-derived cells could be applied in human clinical studies. Pre-evaluation of iPSC cell clones is essential and preclinical transplantation studies would potentially require appropriate primate models.

4 Adipose-Derived Stem Cells as an Alternative Source

Adipose tissue consists predominantly of fibroblasts, endothelial cells, preadipocytes lymphocytes, monocytes, and ADSC (Xu et al. 2003). These latter cells represent a multipotent undifferentiated cell population that is morphologically similar to the mesenchymal stem cells. Accordingly, ADSCs express specific CD surface marker including CD44, CD90, CD105, CD166, and lack the expression of hematopoietic cell markers such as CD34 and CD45 (Bourin et al. 2013). Moreover, ADSCs bear general stem cell markers and possess a proliferation capacity that remains with age (Woo et al. 2016). A spindle-shaped morphology and lack of intracellular lipid droplets characterize the ADSCs in cell culture. Compared with mesenchymal stem cells, ADSCs show more stable morphological features in long-term cell culture and show fewer signs of senescence (Beane et al. 2014).

Based on properties described above, ADSCs have become a promising candidate in the field of peripheral nerve regeneration. Several studies have described the ability of ADSCs and ADSC-derived Schwann cell-like cells to effectively contribute to axonal regeneration following peripheral nerve injury (Fu et al. 2019; Tse et al. 2015; Reid et al. 2011; Santiago et al. 2009) have reported that adipose precursor cells improved nerve regeneration and induced functional recover (Santiago et al. 2009). They also determined the final phenotype of the grafted cells proving that a number of cells contained lipid vacuoles in the newly formed nerve. This finding indicated a portion of grafted cells differentiated into adipocytes rather than Schwann cells. In another interesting study, ADSCs were applied intravenously after crush injury. The ADSCs appeared in the lymphoid organs and restricted the amount of cells migrated into the injured nerve (Marconi et al. 2012). These ADSCs settled in crushed sciatic nerve secreted a considerable amounts of GDNF and IGF-I and reduced the inflammation of the local microenvironment.

A number of studies have used different strategy to differentiate the ADSCs into Schwann cells in vitro to prevent the risk of teratom formation and development of undesired phenotypes (Xu et al. 2008; Kingham et al. 2007; Mantovani et al. 2010; de Luca et al. 2016). These ADSC-derived Schwann cell-like cells expressed myelin proteins, glial markers, and showed similar molecular and functional properties as Schwann cells in cell culture. In vivo studies have also demonstrated that ADSC-derived Schwann cell-like cells had the potential to promote nerve regeneration and functional outcome (di Summa et al. 2010, 2014; Faroni et al. 2011, 2012, 2013a, b). However, the use of conduits appeared to influence significantly the positive effect

of Schwann cell-like ADSCs on nerve regeneration. Robust nerve regeneration and functional outcome could be detected if the cells were seeded in fibrin or silicon conduits (di Summa et al. 2010, 2014). Interestingly, the cells seeded in commercially available collagen-based (Neuragen[®]) conduits have been shown to fail to enhance short-term axon regeneration (di Summa et al. 2014). Reid et al. (2011) have reported in an elegant study that ADSC-derived Schwann cell-like cells have a neuroprotective effect on dorsal root ganglia neurons (Reid et al. 2011). The differentiated ADSC incorporated into a synthetic conduit were able to significantly decrease the apoptotic gene expression in the dorsal root ganglia neurons.

While the present results show that ADSCs would be a promising alternative tools for peripheral nerve repair, further studies are required before clinical translation could be considered. It is important to investigate whether Schwann cell-like ADSCs actively participate in the regeneration process by forming myelin sheaths and supporting endogenous Schwann cells. From the clinical point of view, it would be critical to determine the sufficient number of cells to be used for grafting and the specific subpopulation of ADSCs leading to the best functional outcome.

5 Dental Pulp Stem Cells

Dental pulp stem cells (DPSCs) have been only recently described and characterized by Gronthos and coworkers (Gronthos et al. 2000, 2002; Huang et al. 2009). DPSCs appear to derive from the neural crest (Arthur et al. 2008; Spagnuolo et al. 2018) and accordingly are able to differentiate into neural cells, including Schwann cell-like cells in vitro (Al-Zer et al. 2015; Al-Zer and Kalbounieh 2015; Ullah et al. 2016). These features and their availability from human extracted teeth make them readily useful for human autologous transplantation. Other studies have suggested a wider range of potency to differentiate into neurons, chondrocytes, adipocytes, hepatocytes, and muscle fibers (Iohara et al. 2006; Ishkitiev et al. 2010). Recent findings, based upon the similarity between hair follicle and dental pulp structures and development, suggested that DPSCs may provide a regenerative support to inactive hair follicles. Indeed, when cultured rat and human DPSCs were implanted into surgically inactivated hair follicles, both rodent and human tooth dental cells induced the formation of new epithelial matrix structures and stimulated hair follicle regeneration (Reynolds and Jahoda 2004).

DPSCs express glial fibrillary acidic protein and stem cell markers such as nestin, CD29, CD90, and CD271, but lack hematopoietic markers (Gronthos et al. 2002). They show a great renewal potency, but are also vulnerable to cellular senescence (Morsczeck 2019).

Regarding their regenerative abilities, intravitreally grafted DPSCs were able to promote axonal regeneration and survival of retinal ganglion cells after optic nerve injury (Mead et al. 2013). In vitro studies have also suggested that these cells may support the regenerative capacity of injured peripheral axons. In DRG-DPSC co-cultures, the dental stem cells differentiated into Schwann cells and guided the DRG axons along a collagen construct in an aligned pattern (Martens et al. 2014). DPSCs proved to be effective in a peripheral nerve injury model, too.

Transplantation of DPSCs into a transected peripheral nerve reportedly induced enhanced myelination and improved functional recovery (Carnevale et al. 2018).

In conclusion, it can be stated that DPSCs on one hand represent a relatively easily available autologous stem cell source and they proved to possess a considerable regenerative potential in several injury models, including peripheral nerve injuries (Mead et al. 2017).

6 Hair Follicle-Associated Stem Cells

Hair follicle-associated stem cells (HFASCs) reside adjacent to the hair follicle, superficial to the dermal papilla. They possess common features with embryonic neural crest stem cells and express the ESC markers Nanog and Oct4, too. They are considered neural crest stem cell-like pluripotent stem cells with an ability to differentiate into glial (especially Schwann cells) and neuronal cell lineages, keratinocytes, melanocytes, smooth and cardiac muscle fibers (Hu et al. 2007; Amoh et al. 2009a; Yashiro et al. 2015; Sieber-Blum et al. 2004).

Transplantation of undifferentiated HFASCs into the lesion site of crushed or transected peripheral nerves promoted the differentiation of the HFASCs into Schwann cells which then were able to myelinate naked axons and induce some functional recovery in mouse models (Amoh et al. 2005, 2009b, 2012). On the other hand, grafting of HFASC-derived (i.e., predifferentiated cells) neurons and Schwann cells into an acellular conduit resulted in myelination of axons which appeared to regenerate, and the functional outcome (based only on conduction velocity measurements) was weaker than in the untreated group (Lin et al. 2009).

In another experimental set-up, HFASCs seeded in polyvinylidene fluoride membranes were used to bridge a mouse sciatic nerve gap injury. The implanted cells differentiated into neurons (47%) and GFAP+ glial cells (8%) and appeared to contact the proximal and distal nerve stumps. However, as myelination of regenerating host axons from the proximal stump was not demonstrated, the improved functional data could not be associated with histological features (Yamazaki et al. 2017).

7 Conclusions

Although peripheral nerve injuries can be much more successfully treated than CNS ones, the recovery rate after successful surgical coaptation/grafting procedures is still not as good as expected. Various stem cell-based experimental therapies provide evidence that they are able to positively influence the regenerative environment after PNS injury. These promising experimental data suggest a potential future role for stem cell-based treatments that may further improve the morphological and functional recovery after nerve injury.

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References

- Abdallah BM, Kassem M (2008) Human mesenchymal stem cells: from basic biology to clinical applications. *Gene Ther* 15:109–116. <https://doi.org/10.1038/sj.gt.3303067>
- Al-Zer H, Kalboun H (2015) Dental pulp stem cells-derived Schwann cells for peripheral nerve injury regeneration. *Neural Regen Res* 10:1945–1946. <https://doi.org/10.4103/1673-5374.172309>
- Al-Zer H et al (2015) Enrichment and Schwann cell differentiation of neural crest-derived dental pulp stem cells. *In Vivo* 29:319–326
- Amoh Y et al (2005) Implanted hair follicle stem cells form Schwann cells that support repair of severed peripheral nerves. *Proc Natl Acad Sci USA* 102:17734–17738. <https://doi.org/10.1073/pnas.0508440102>
- Amoh Y et al (2009a) Human hair follicle pluripotent stem (hfPS) cells promote regeneration of peripheral-nerve injury: an advantageous alternative to ES and iPS cells. *J Cell Biochem* 107:1016–1020. <https://doi.org/10.1002/jcb.22204>
- Amoh Y et al (2009b) Human and mouse hair follicles contain both multipotent and monopotent stem cells. *Cell Cycle* 8:176–177. <https://doi.org/10.4161/Cc.8.1.7342>
- Amoh Y et al (2012) Nestin-positive hair follicle pluripotent stem cells can promote regeneration of impinged peripheral nerve injury. *J Dermatol* 39:33–38. <https://doi.org/10.1111/j.1346-8138.2011.01413.x>
- Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S (2008) Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. *Stem Cells* 26:1787–1795. <https://doi.org/10.1634/stemcells.2007-0979>
- Beane OS, Fonseca VC, Cooper LL, Koren G, Darling EM (2014) Impact of aging on the regenerative properties of bone marrow-, muscle-, and adipose-derived mesenchymal stem/stromal cells. *PLoS One* 9:ARTN e115963. <https://doi.org/10.1371/journal.pone.0115963>
- Bhangra KS, Busuttill F, Phillips JB, Rahim AA (2016, 2016) Using stem cells to grow artificial tissue for peripheral nerve repair. *Stem Cells Int:Artn* 7502178. <https://doi.org/10.1155/2016/7502178>
- Bianco P, Robey PG (2015) Skeletal stem cells. *Development* 142:1023–1027. <https://doi.org/10.1242/dev.102210>
- Bourin P et al (2013) Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy* 15:641–648. <https://doi.org/10.1016/j.jcyt.2013.02.006>
- Carnevale G et al (2018) Human dental pulp stem cells expressing STRO-1, c-kit and CD34 markers in peripheral nerve regeneration. *J Tissue Eng Regen Med* 12:E774–E785. <https://doi.org/10.1002/term.2468>
- Caseiro AR, Pereira T, Ivanova G, Luis AL, Mauricio AC (2016, 2016) Neuromuscular regeneration: perspective on the application of mesenchymal stem cells and their secretion products. *Stem Cells Int:Artn* 9756973. <https://doi.org/10.1155/2016/9756973>
- Chen CJ et al (2007) Transplantation of bone marrow stromal cells for peripheral nerve repair. *Exp Neurol* 204:443–453. <https://doi.org/10.1016/j.expneurol.2006.12.004>
- Crisan M et al (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 3:301–313. <https://doi.org/10.1016/j.stem.2008.07.003>
- de Luca AC, Faroni A, Downes S, Terenghi G (2016) Differentiated adipose-derived stem cells act synergistically with RGD-modified surfaces to improve neurite outgrowth in a co-culture model. *J Tissue Eng Regen Med* 10:647–655. <https://doi.org/10.1002/term.1804>
- Deng WW, Obrocka M, Fischer I, Prockop DJ (2001) In vitro differentiation of human marrow stromal cells into early progenitors of neural cells by conditions that increase intracellular cyclic AMP. *Biochem Biophys Res Commun* 282:148–152. <https://doi.org/10.1006/bbrc.2001.4570>
- Dezawa M, Takahashi I, Esaki M, Takano M, Sawada H (2001) Sciatic nerve regeneration in rats induced by transplantation of in vitro differentiated bone-marrow stromal cells. *Eur J Neurosci* 14:1771–1776. <https://doi.org/10.1046/j.0953-816x.2001.01814.x>

- di Summa PG et al (2010) Adipose-derived stem cells enhance peripheral nerve regeneration. *J Plast Reconstr Aes* 63:1544–1552. <https://doi.org/10.1016/j.bjps.2009.09.012>
- di Summa PG, Kingham PJ, Campisi CC, Raffoul W, Kalbermatten DF (2014) Collagen (NeuraGen (R)) nerve conduits and stem cells for peripheral nerve gap repair. *Neurosci Lett* 572:26–31. <https://doi.org/10.1016/j.neulet.2014.04.029>
- Eglitis MA, Mezey E (1997) Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc Natl Acad Sci USA* 94:4080–4085. <https://doi.org/10.1073/pnas.94.8.4080>
- Fairbairn NG, Meppelink AM, Ng-Glazier J, Randolph MA, Winograd JM (2015) Augmenting peripheral nerve regeneration using stem cells: a review of current opinion. *World J Stem Cells* 7:11–26. <https://doi.org/10.4252/wjsc.v7.i1.11>
- Faroni A et al (2011) Schwann-like adult stem cells derived from bone marrow and adipose tissue express gamma-aminobutyric acid type B receptors. *J Neurosci Res* 89:1351–1362. <https://doi.org/10.1002/jnr.22652>
- Faroni A, Terenghi G, Magnaghi V (2012) Expression of functional gamma-aminobutyric acid type A receptors in Schwann-like adult stem cells. *J Mol Neurosci* 47:619–630. <https://doi.org/10.1007/s12031-011-9698-9>
- Faroni A, Calabrese F, Riva MA, Terenghi G, Magnaghi V (2013a) Baclofen modulates the expression and release of neurotrophins in Schwann-like adipose stem cells. *J Mol Neurosci* 49:233–243. <https://doi.org/10.1007/s12031-012-9813-6>
- Faroni A, Terenghi G, Reid AJ (2013b) Adipose-derived stem cells and nerve regeneration: promises and pitfalls. *Int Rev Neurobiol* 108:121. <https://doi.org/10.1016/B978-0-12-410499-0.00005-8>
- Fu XM et al (2019) The combination of adipose-derived Schwann-like cells and acellular nerve allografts promotes sciatic nerve regeneration and repair through the JAK2/STAT3 signaling pathway in rats. *Neuroscience* 422:134–145. <https://doi.org/10.1016/j.neuroscience.2019.10.018>
- Fusaki N, Ban H, Nishiyama A, Saeki K, Hasegawa M (2009) Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. *Proc Jpn Acad B-Phys* 85:348–362. <https://doi.org/10.2183/pjab.85.348>
- Grochmal J, Dhaliwal S, Stys PK, van Minnen J, Midha R (2014) Skin-derived precursor Schwann cell myelination capacity in focal tibial demyelination. *Muscle Nerve* 50:262–272. <https://doi.org/10.1002/mus.24136>
- Gronthos S, Mankani M, Brahimi J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci USA* 97:13625–13630. <https://doi.org/10.1073/pnas.240309797>
- Gronthos S et al (2002) Stem cell properties of human dental pulp stem cells. *J Dent Res* 81:531–535. <https://doi.org/10.1177/154405910208100806>
- Hanna J et al (2007) Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science* 318:1920–1923. <https://doi.org/10.1126/science.1152092>
- Hu J, Zhu QT, Liu XL, Xu YB, Zhu JK (2007) Repair of extended peripheral nerve lesions in rhesus monkeys using acellular allogenic nerve grafts implanted with autologous mesenchymal stem cells. *Exp Neurol* 204:658–666. <https://doi.org/10.1016/j.expneurol.2006.11.018>
- Huang GTJ, Gronthos S, Shi S (2009) Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. *J Dent Res* 88:792–806. <https://doi.org/10.1177/0022034509340867>
- Iohara K et al (2006) Side population cells isolated from porcine dental pulp tissue with self-renewal and multipotency for dentinogenesis, chondrogenesis, adipogenesis, and neurogenesis. *Stem Cells* 24:2493–2503. <https://doi.org/10.1634/stemcells.2006-0161>
- Ishkitiev N et al (2010) Deciduous and permanent dental pulp mesenchymal cells acquire hepatic morphologic and functional features in vitro. *J Endod* 36:469–474. <https://doi.org/10.1016/j.joen.2009.12.022>

- Kim D et al (2009) Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell* 4:472–476. <https://doi.org/10.1016/j.stem.2009.05.005>
- Kingham PJ et al (2007) Adipose-derived stem cells differentiate into a Schwann cell phenotype and promote neurite outgrowth in vitro. *Exp Neurol* 207:267–274. <https://doi.org/10.1016/j.expneurol.2007.06.029>
- Lin HY et al (2009) Pluripotent hair follicle neural crest stem-cell-derived neurons and Schwann cells functionally repair sciatic nerves in rats. *Mol Neurobiol* 40:216–223. <https://doi.org/10.1007/s12035-009-8082-z>
- Liu QY et al (2012) Human neural crest stem cells derived from human ESCs and induced pluripotent stem cells: induction, maintenance, and differentiation into functional Schwann cells. *Stem Cells Transl Med* 1:266–278. <https://doi.org/10.5966/sctm.2011-0042>
- Ma MS, Boddeke E, Copray S (2015) Pluripotent stem cells for Schwann cell engineering. *Stem Cell Rev Rep* 11:205–218. <https://doi.org/10.1007/s12015-014-9577-1>
- Maherali N et al (2008) A high-efficiency system for the generation and study of human induced pluripotent stem cells. *Cell Stem Cell* 3:340–345. <https://doi.org/10.1016/j.stem.2008.08.003>
- Mantovani C et al (2010) Bone marrow- and adipose-derived stem cells show expression of myelin mRNAs and proteins. *Regen Med* 5:403–410. <https://doi.org/10.2217/Rme.10.15>
- Marconi S et al (2012) Human adipose-derived mesenchymal stem cells systemically injected promote peripheral nerve regeneration in the mouse model of sciatic crush. *Tissue Eng Pt A* 18:1264–1272. <https://doi.org/10.1089/ten.tea.2011.0491>
- Martens W et al (2014) Human dental pulp stem cells can differentiate into Schwann cells and promote and guide neurite outgrowth in an aligned tissue-engineered collagen construct in vitro. *FASEB J* 28:1634–1643. <https://doi.org/10.1096/fj.13-243980>
- Mead B, Logan A, Berry M, Leadbeater W, Scheven BA (2013) Intravitreally transplanted dental pulp stem cells promote neuroprotection and axon regeneration of retinal ganglion cells after optic nerve injury. *Invest Ophthalmol Vis Sci* 54:7544–7556. <https://doi.org/10.1167/iovs.13-13045>
- Mead B, Logan A, Berry M, Leadbeater W, Scheven BA (2017) Concise review: dental pulp stem cells: a novel cell therapy for retinal and central nervous system repair. *Stem Cells* 35:61–67. <https://doi.org/10.1002/stem.2398>
- Morszeck C (2019) Cellular senescence in dental pulp stem cells. *Arch Oral Biol* 99:150–155. <https://doi.org/10.1016/j.archoralbio.2019.01.012>
- Narazaki G et al (2008) Directed and systematic differentiation of cardiovascular cells from mouse induced pluripotent stem cells. *Circulation* 118:498–506. <https://doi.org/10.1161/Circulationaha.108.769562>
- Okita K, Ichisaka T, Yamanaka S (2007) Generation of germline-competent induced pluripotent stem cells. *Nature* 448:313–317. <https://doi.org/10.1038/nature05934>
- Pepper JP, Wang TV, Hennes V, Sun SY, Ichida JK (2017) Human induced pluripotent stem cell-derived motor neuron transplant for neuromuscular atrophy in a mouse model of sciatic nerve injury. *JAMA Facial Plast Surg* 19:197–205. <https://doi.org/10.1001/jamafacial.2016.1544>
- Polak JM, Bishop AE (2006) Stem cells and tissue engineering: past, present, and future. *Ann N Y Acad Sci* 1068:352–366. <https://doi.org/10.1196/annals.1346.001>
- Reid AJ et al (2011) Nerve repair with adipose-derived stem cells protects dorsal root ganglia neurons from apoptosis. *Neuroscience* 199:515–522. <https://doi.org/10.1016/j.neuroscience.2011.09.064>
- Reynolds AJ, Jahoda CAB (2004) Cultured human and rat tooth papilla cells induce hair follicle regeneration and fiber growth. *Differentiation* 72:566–575. <https://doi.org/10.1111/j.1432-0436.2004.07209010.x>
- Rodrigues MCO, Rodrigues AA, Glover LE, Voltarelli J, Borlongan CV (2012) Peripheral nerve repair with cultured Schwann cells: getting closer to the clinics. *Sci World J:Artn* 413091. <https://doi.org/10.1100/2012/413091>
- Santiago LY, Clavijo-Alvarez J, Brayfield C, Rubin JP, Marra KG (2009) Delivery of adipose-derived precursor cells for peripheral nerve repair. *Cell Transplant* 18:145–158. <https://doi.org/10.3727/096368909788341289>

- Sieber-Blum M, Grim M, Hu YF, Szeder V (2004) Pluripotent neural crest stem cells in the adult hair follicle. *Dev Dyn* 231:258–269. <https://doi.org/10.1002/dvdy.20129>
- Si-Tayeb K et al (2010) Generation of human induced pluripotent stem cells by simple transient transfection of plasmid DNA encoding reprogramming factors. *BMC Dev Biol* 10:Artm 81. <https://doi.org/10.1186/1471-213x-10-81>
- Somers A et al (2010) Generation of transgene-free lung disease-specific human induced pluripotent stem cells using a single excisable lentiviral stem cell cassette. *Stem Cells* 28:1728–1740. <https://doi.org/10.1002/stem.495>
- Sowa Y et al (2016) Adipose-derived stem cells promote peripheral nerve regeneration in vivo without differentiation into Schwann-Like lineage. *Plast Reconstr Surg* 137:318e–330e. <https://doi.org/10.1097/01.prs.0000475762.86580.36>
- Spagnuolo G et al (2018) Commitment of oral-derived stem cells in dental and maxillofacial applications. *Dent J (Basel)* 6. <https://doi.org/10.3390/dj6040072>
- Stadtfield M, Brennand K, Hochedlinger K (2008a) Reprogramming of pancreatic beta cells into induced pluripotent stem cells. *Curr Biol* 18:890–894. <https://doi.org/10.1016/j.cub.2008.05.010>
- Stadtfield M, Nagaya M, Utikal J, Weir G, Hochedlinger K (2008b) Induced pluripotent stem cells generated without viral integration. *Science* 322:945–949. <https://doi.org/10.1126/science.1162494>
- Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663–676. <https://doi.org/10.1016/j.cell.2006.07.024>
- Takahashi K et al (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131:861–872. <https://doi.org/10.1016/j.cell.2007.11.019>
- Tse KH, Novikov LN, Wiberg M, Kingham PJ (2015) Intrinsic mechanisms underlying the neurotrophic activity of adipose derived stem cells. *Exp Cell Res* 331:142–151. <https://doi.org/10.1016/j.yexcr.2014.08.034>
- Tsuiji O et al (2010) Therapeutic potential of appropriately evaluated safe-induced pluripotent stem cells for spinal cord injury. *Proc Natl Acad Sci USA* 107:12704–12709. <https://doi.org/10.1073/pnas.0910106107>
- Uemura T et al (2012) Transplantation of induced pluripotent stem cell-derived neurospheres for peripheral nerve repair. *Biochem Bioph Res Co* 419:130–135. <https://doi.org/10.1016/j.bbrc.2012.01.154>
- Ullah I et al (2016) In vitro comparative analysis of human dental stem cells from a single donor and its neuronal differentiation potential evaluated by electrophysiology. *Life Sci* 154:39–51. <https://doi.org/10.1016/j.lfs.2016.04.026>
- Wang AJ et al (2011) Induced pluripotent stem cells for neural tissue engineering. *Biomaterials* 32:5023–5032. <https://doi.org/10.1016/j.biomaterials.2011.03.070>
- Wang Y, Li ZW, Luo M, Li YJ, Zhang KQ (2015) Biological conduits combining bone marrow mesenchymal stem cells and extracellular matrix to treat long-segment sciatic nerve defects. *Neural Regen Res* 10:965–971. <https://doi.org/10.4103/1673-5374.158362>
- Woo DH, Hwang HS, Shim JH (2016) Comparison of adult stem cells derived from multiple stem cell niches. *Biotechnol Lett* 38:751–759. <https://doi.org/10.1007/s10529-016-2050-2>
- Woodbury D, Schwarz EJ, Prockop DJ, Black IB (2000) Adult rat and human bone marrow stromal cells differentiate into neurons. *J Neurosci Res* 61:364–370. [https://doi.org/10.1002/1097-4547\(20000815\)61:4<364::Aid-Jnr2>3.0.Co;2-C](https://doi.org/10.1002/1097-4547(20000815)61:4<364::Aid-Jnr2>3.0.Co;2-C)
- Xu HY et al (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112:1821–1830. <https://doi.org/10.1172/Jci200319451>
- Xu YF et al (2008) Myelin-forming ability of Schwann cell-like cells induced from rat adipose-derived stem cells in vitro. *Brain Res* 1239:49–55. <https://doi.org/10.1016/j.brainres.2008.08.088>
- Xu D et al (2009) Phenotypic correction of murine hemophilia A using an iPS cell-based therapy. *Proc Natl Acad Sci USA* 106:808–813. <https://doi.org/10.1073/pnas.0812090106>

- Yamanaka S (2020) Pluripotent stem cell-based cell therapy-promise and challenges. *Cell Stem Cell* 27:523–531. <https://doi.org/10.1016/j.stem.2020.09.014>
- Yamazaki A et al (2017) Implanted hair-follicle-associated pluripotent (HAP) stem cells encapsulated in polyvinylidene fluoride membrane cylinders promote effective recovery of peripheral nerve injury. *Cell Cycle* 16:1927–1932. <https://doi.org/10.1080/15384101.2017.1363941>
- Yashiro M et al (2015) From hair to heart: nestin-expressing hair-follicle-associated pluripotent (HAP) stem cells differentiate to beating cardiac muscle cells. *Cell Cycle* 14:2362–2366. <https://doi.org/10.1080/15384101.2015.1042633>
- Yoshida Y, Yamanaka S (2010) Recent stem cell advances: induced pluripotent stem cells for disease modeling and stem cell-based regeneration. *Circulation* 122:80–87. <https://doi.org/10.1161/Circulationaha.109.881433>
- Yu JY et al (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318:1917–1920. <https://doi.org/10.1126/science.1151526>
- Zarbaksh S et al (2012) The effects of Schwann and bone marrow stromal stem cells on sciatic nerve injury in rat: a comparison of functional recovery. *Cell J* 14:39–46
- Zhou WB, Freed CR (2009) Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. *Stem Cells* 27:2667–2674. <https://doi.org/10.1002/stem.201>
- Zhou HY et al (2009) Generation of induced pluripotent stem cells using recombinant proteins (vol 4, pg 381, 2009). *Cell Stem Cell* 4:581–581. <https://doi.org/10.1016/j.stem.2009.05.014>

Extracellular Vesicles for Nerve Regeneration

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Abstract

The rapidly evolving collective fields of tissue engineering and regenerative medicine (TERM) have high potential for development of new clinical therapies for treatment of peripheral nerve injuries. The positive results of experimental nerve repair strategies incorporating transplanted cells are now largely attributed to the secretion of paracrine factors, rather than cell engraftment. Extracellular

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vesicles (EVs), which make up part of the cell secretome, have emerged as important intercellular communicators in various biological processes. The bioactivity of EVs is mediated by their cargoes of various types of nucleic acids and proteins. In this chapter we present an overview of the biogenesis, isolation, and characterization of EVs, and describe how EVs might be useful adjuncts in TERM for peripheral nerve repair. In summary, EVs enhance axonal growth *in vitro* and *in vivo*, modulate Schwann cell function, and are highly likely to influence the neuroimmune and vascular cell reactions activated by peripheral nerve injury. Thus EVs have high potential for use as a new cell-free therapeutic approach for nerve repair.

1 Introduction

Peripheral nerve injury occurs commonly as a result of domestic, industrial, or military trauma, and also may arise during birth complications. Predominantly associated with upper limb trauma, the incidence rate is approximately 17 per 100,000 persons, mostly adults of working age (Tapp et al. 2019). For the affected individuals, sensory and motor functions are impaired as a consequence of the disconnection of the normal signaling pathways between the central nervous system and the peripheral target structures. The severity and position of the trauma, the age of the patient, and the type of nerves involved all influence how likely functional recovery will be, and in a significant number of cases the injury results in permanent disability (Siemionow and Brzezicki 2009). For most injuries there is a need to perform some type of surgical repair, this often involves tensionless suturing of the outermost connective tissue layer of the nerve, the epineurium, to reconnect the proximal and distal segments. In the cases of nerve gap, where end-end suturing is not possible, a nerve graft is used to bridge the gap (Siemionow and Brzezicki 2009). This is far from ideal since it requires the sacrifice of healthy nerves and for extensive surgeries there is only a finite supply of suitable nerves. The surgical approaches aim to assist the endogenous processes of axonal regrowth, which allow for some degree of regeneration in the PNS. In response to injury, molecular and cellular changes occur within the neuronal cell bodies and the target structures, and there is an active remodeling at the trauma site, with the distal segment undergoing a process known as Wallerian degeneration (Rotshenker 2011). The transformation of the PNS glial cells, the Schwann cells, toward a repair phenotype, together with immune cell infiltration and vascular cell responses allows for the regrowth of axons at a rate of approximately 1–2 mm per day. This means that for distal injuries, for example, in digital nerves, regeneration should be sufficient to reverse any temporary loss of sensation to the finger-tips. In contrast, proximal brachial plexus injuries are functionally devastating. Prolonged denervation leads to chronic loss of growth factor support for the neurons which as a consequence may die. Primary sensory neurons appear to be more likely to undergo apoptosis than spinal motoneurons, with 40% of dorsal root ganglion (DRG) neurons dying following injury (Hart et al. 2008). Distally, prolonged denervation of the target muscles leads to irreversible muscle fiber atrophy (Gutmann 1962).

Surgical repair methods, which are largely unchanged during the recent decades, are clearly not enough to overcome the limitations of the body's own response to peripheral nerve injury and alternative or complementary approaches are needed. Traditional pharmacological approaches, building on our knowledge of the complex cellular and molecular events of peripheral nerve regeneration might play a role. For example, N-acetyl cysteine, a well-established clinically safe drug, has been shown to protect DRG neurons after peripheral nerve injury (West et al. 2007). However, the rapidly evolving collective fields of tissue engineering and regenerative medicine (TERM) have high potential for overcoming the problems of peripheral nerve injury. The basic principles of TERM are to use materials and cells to recreate missing tissue, contribute to tissue healing, and modulate the body's innate regenerative reactions. Various materials have been used in the construction of nerve guidance conduits (NGCs) which are used to entubulate the damaged proximal and distal nerve ends. Although a number of NGCs are commercially available, they are only suitable for the bridging of small nerve gaps and their relatively simple structure means their performance is inferior to nerve grafts (Kehoe et al. 2012). Structural modifications, such as addition of topographic guiding filaments and fibers, have shown some benefits in experimental models but combination with biological agents such as specific growth factors or ex vivo expanded cells is likely to also be necessary for the optimization of NGCs (Carvalho et al. 2019).

Schwann cells, as the endogenous regulators of axon regeneration, have been proposed as the most obvious choice for use in cell therapy for peripheral nerve injuries. However, the need for sacrifice of donor nerve, low cell yields, and initial slow culture expansion times limits their clinical application. Alternatively, embryonic stem cells could be differentiated into Schwann cells but there are ethical implications and doubts still persist concerning transplantation of any undifferentiated cells which could lead to tumor formation. Therefore, as somewhat of a middle ground, the use of mesenchymal stem cells (MSCs) has increased significantly. Numerous in vitro studies have shown that MSCs from various tissue sources can differentiate into a Schwann cell-like phenotype (Wakao et al. 2014) but only a limited number of studies suggest that in vivo transplanted MSCs assume markers of Schwann cells (Tohill et al. 2004). Furthermore, MSCs express a wide range of growth factors produced by Schwann cells (Kolar and Kingham 2014). Thus there is now a wealth of literature which shows that NGCs combined with MSCs therapy can facilitate peripheral nerve repair (Ashraf et al. 2019) but the mechanisms by which they do this are still not completely clear. The limited engraftment and poor survival of transplanted MSCs (Ezquer et al. 2017) suggests that their mode of action might not primarily be that of differentiation and direct rebuilding of damaged tissue as originally proposed. Rather, the therapeutic effects of MSCs can be attributed to indirect effects of modulation of the endogenous molecular and cellular regenerative reactions, via secreted factors. The so-called cell secretome has been shown to recapitulate the effects of the parent cells. For example, cell secretome is neuroprotective (Grinblat et al. 2018), enhances nerve repair when applied in a conduit (Sugimura-Wakayama et al. 2015), and alleviates diabetic and neuropathic pain (Brini et al. 2017; Gama et al. 2018). Depending on the

parent cell type, the secretome comprises a multitude of soluble molecules such as neurotrophic growth factors, angiogenic molecules, chemokines, cytokines, and other immunomodulatory factors which all act in a classic paracrine manner. Additionally, factors produced by the cells can be found encapsulated in cell-secreted vesicles known as extracellular vesicles (EVs). The relative importance of the various secreted components for peripheral nerve repair remains to be fully elucidated. In this chapter we focus on the role of EVs as modulators of nerve regeneration and describe their potential use for clinical application in treatment of peripheral nerve injuries.

2 Biogenesis and Uptake Mechanisms of EVs

Secreted by numerous cell types, EVs are phospholipid bilayer membrane-enclosed structures which carry biomolecules originating from the parent cell (Fig. 1). EVs are classified into subcategories (Akers et al. 2013). Small size EVs, commonly known as exosomes, are vesicles ranging in size from 30 to 150 nm whereas microvesicles/ectosomes are large vesicles up to 1000 nm. Although exomeres are formed of non-membrane bound nanoparticles and other macromolecular complexes, they are also sometimes included under the collective term of EVs (Zhang et al. 2018). At the other end of the scale, apoptotic bodies are large size vesicles (1–5 μ m) which are only released during cell apoptosis. Exosomes are formed through inward budding of the cell membrane and the formation of multivesicular endosomes, which capture exosomes and then fuse with the cell membrane and release exosomes through exocytosis (Thery 2011). In contrast, microvesicles are formed through outward budding of the cell membrane (Thery et al. 2018). The intracellular sorting machinery responsible for formation of the EVs is complex but for both exosomes and microvesicles this involves clustering of protein and lipids in the membranes and internal sequestration of nucleic acids, proteins and other bioactive molecules and metabolites (Fig. 1). The relative involvement of endosomal sorting complex required for transport (ESCRT) dependent and ESCRT-independent pathways determines the selection of molecules associated with the respective EVs. The ESCRT-independent pathways are responsible for clustering of tetraspanins such as CD63, CD81, and CD9 and inducing inward budding of vesicles. These molecules are often used to characterize preparations of EVs as exosomes (Dong et al. 2019), although they have also been detected in microsomes (Crescitelli et al. 2013). In contrast, calcium-dependent mechanisms are important for microvesicle formation, and they are characterized by a range of other integrin and selectin markers such as CD40 ligand and annexin A1 (Dong et al. 2019). Nucleic acids, including DNA, mRNA, microRNAs (miRNAs), ribosomal RNAs, and long noncoding RNAs, are important cargoes of EVs. The nucleic acids are protected by the EVs bilayer membrane thus ensuring that they are not degraded and therefore can be transported over long distances (Eldh et al. 2010). Although the miRNAs represent only a relatively small fraction of the EVs cargo, there are now a multitude of studies indicating their role as mediators of the reactions in the recipient cells (Bjorge et al. 2017).

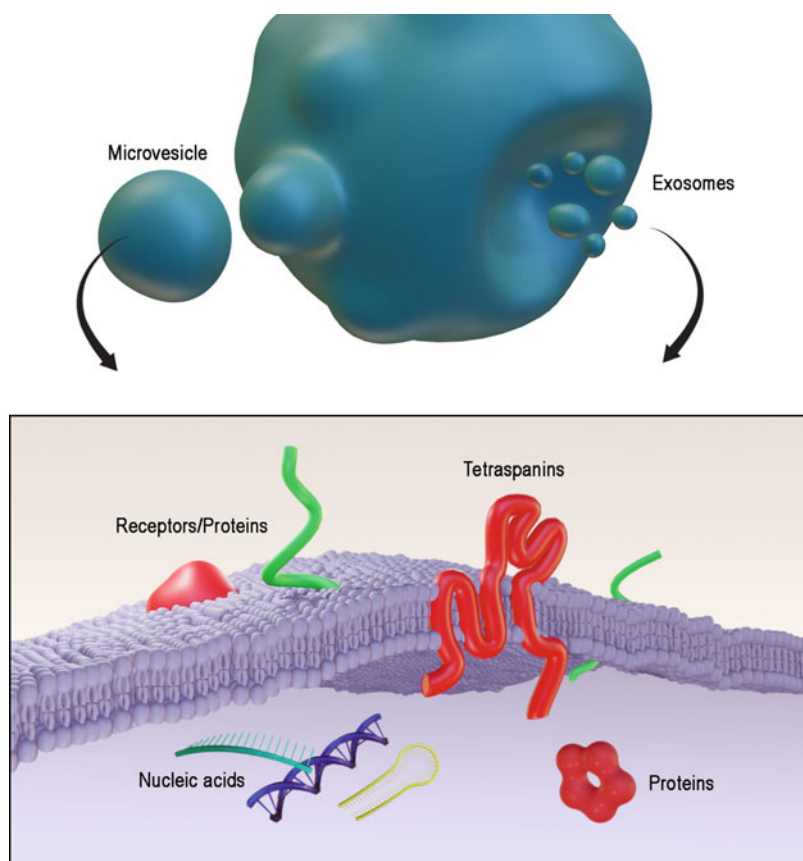


Fig. 1 Overview of extracellular vesicles biology. Microvesicles (100–1000 nm) are created through outward budding of the cell membrane whereas exosome (30–150 nm) formation involves inward budding and the formation of multivesicular endosomes. Extracellular vesicle cargo consists predominantly of various types of nucleic acids and proteins which are encapsulated by a lipid bilayer membrane containing tetraspanins and various other membrane proteins and receptors

Differential sorting of miRNAs into EVs is controlled, in large part, by their specific sequences (Gibbins et al. 2009). Further work needs to be done to fully characterize the biogenesis and sorting mechanisms of the EVs. The multiple pathways involved result in the cellular release of a highly heterogeneous population of EVs, for which it is difficult to assign specific mechanisms to specific subpopulations.

Internalization of EVs appears to be necessary for their function as intercellular communicators. Various mechanisms have been suggested including clathrin-mediated endocytosis, phagocytosis, macropinocytosis, and direct membrane fusion (Mulcahy et al. 2014). Fluorescent lipid membrane binding dyes such as PKH26 are commonly used to stain the EVs and monitor their cellular uptake. Alternatively, EV-enriched proteins such as the tetraspanins can be tagged with green fluorescent protein. Staining, used together with chemical inhibitors or specific antibodies, has shed light on some of

the protein interactions necessary for endocytosis. These proteins include the tetraspanins, integrins, immunoglobulins, proteoglycans, and lectins (Mulcahy et al. 2014). *In vitro* experiments show that endocytotic uptake of EVs is often rapid (as early as 15 min after application to recipient cells) and is an active, energy-dependent process (Feng et al. 2010; Morelli et al. 2004). It is likely that a cell uptakes EVs via multiple endocytic mechanisms (Mulcahy et al. 2014). Clathrin-coated vesicles, once inside the cell, are uncoated and then fuse with the endosome and subsequently deposit their contents. The use of chlorpromazine which prevents formation of clathrin-coated pits at the plasma membrane has shown the importance of this as one mechanism of EVs uptake (Wang et al. 1993). It is also clear that there are clathrin-independent mechanisms such as caveolin-dependent endocytosis involved in EVs uptake (Nanbo et al. 2013). Other mechanisms might be more cell specific. Receptor-mediated phagocytosis is used by macrophages to internalize large particles; however, it has been suggested that the smaller EVs can also be internalized via this route and the PI3K inhibitors wortmannin and LY294002 are commonly used to determine the role of phagocytosis (Stephens et al. 2002). Macropinocytosis, which shares some similarities to the phagocytic mechanism, might also play a minor role in internalization of EVs. The majority of studies have indicated any of these aforementioned forms of endocytosis to be important for EVs uptake but it is also possible that direct membrane fusion occurs between compatible regions on the surface of the EVs (with lipid-raft like composition) and the recipient cell plasma membrane (Mulcahy et al. 2014). Whether or not EVs uptake is a cell-specific process is still not completely clear. Some studies suggest that the recipient cell and EVs must share the corresponding ligand and receptors whereas other studies show identical EVs can be up taken by any cell type. Thus it is likely that there are multiple mechanisms responsible for the EVs role in cell communication throughout different tissues of the body. Once inside the cell, the cargo of the EVs can interact with the signaling pathways of the cell, influencing processes such as cell proliferation, differentiation and transcription, apoptosis, migration, and immune reactions.

3 EVs Isolation

A variety of laboratory methods have been used to isolate EVs for preclinical studies, but more recently, new technologies have been developed with the view toward large-scale manufacturing for clinical application. The traditional technique is to use ultracentrifugation to separate EVs from other cell-secreted components based on their density. This produces preparations with low protein contamination but requires multiple steps to fractionate the different subpopulations of EVs and has the disadvantage that it is low throughput. Size exclusion chromatography separates EVs on the basis of hydrodynamic volume and utilizes inexpensive columns to yield relatively clean preparations compared with ultracentrifugation. Filtration uses membranes with specific pore sizes whereas immunoaffinity-based isolation strategies capture EVs using specific antibodies. For large-scale production under good manufacturing practice (GMP) conditions, tangential flow filtration coupled with chromatography-based methods has been developed (Watson et al. 2018). With the rapid growth in basic preclinical studies of EVs a number of commercially available

kits have become available. These are based on polymer precipitation, simple column separation, and magnetic labeling methods which yield EVs with similar size distributions to ultracentrifugation (Helwa et al. 2017) but they can result in a high level of protein contamination. New technologies are also being used for isolation of EVs. For example, label-free microfluidic devices can be used to sort EVs based on their viscoelasticity and acoustic properties (Guo et al. 2018).

EVs have been isolated from various biological fluids but in the context of TERM it is important to consider the choice of cellular source and culture conditions which will enable the production of suitable quantities of clinical grade vesicles. Secretion of EVs is influenced by cell density, cell passage number (and donor cell age), both soluble factors in the culture media and the attachment substrata, and oxygen levels. Simple 2D culture involves growing cells in tissue culture flasks in a serum-free medium or, if necessary, using exosome-depleted serum as supplement and then collecting the medium after 24–72 h. It is important to note that when adapting cell culture conditions, switching to the EVs-depleted medium can itself affect cell growth and stem cell differentiation potential. Use of 3D scaffolds, which create more physiologically relevant conditions, has been shown to influence the quantity and quality of secreted EVs (Jangamreddy et al. 2018). For nonporous scaffolds such as hydrogels it is more difficult to retrieve the EVs and they may be damaged during processing of the scaffold. For large-scale EVs production, hollow fiber bioreactor technology has been developed. The high surface area of bundled cylindrical hollow fibers permits billions of cells to be cultured, versus the millions of cells which can be grown in T175 flasks. With media continually flowing through these bioreactors it is possible to collect 4–40 fold more EVs than in traditional culture flasks (Patel et al. 2018; Watson et al. 2016). EVs should be harvested from sub-confluent cultures, especially for cells such as MSCs which undergo senescence at confluence. High density cultures of MSCs produce significantly lower amounts of EVs, although the bioactivity of the EVs might not be negatively impacted (Patel et al. 2017). However, late passage MSCs do secrete EVs with reduced bioactivity (Patel et al. 2017). To overcome this problem, immortalized MSCs have been used for the production of EVs (Krawczenko et al. 2020). Differentiation status of cells can also influence the bioactivity of EVs and this may be reflected by changes in their cargo. For example, exosomes obtained from Schwann cell-like differentiated adipose stem cells enhance neurite outgrowth whereas EVs from undifferentiated stem cells, which express lower levels of neurogenic mRNAs and miRNAs, do not (Ching et al. 2018). Topography and stiffness of the culture matrix can also affect the cargo composition of the EVs (Patel et al. 2018).

4 Preclinical Research on EVs for Nerve Regeneration

4.1 EVs for Axon Regeneration and Neuroprotection

A number of *in vitro* and *in vivo* studies have shown how EVs can enhance axon outgrowth and regeneration. After peripheral nerve injury, Schwann cells dedifferentiate and assume a repair/regenerative phenotype which mediates axon regeneration. Exosomes from de-differentiated Schwann cells promote dorsal root ganglia

(DRG) in vitro neurite outgrowth by inhibiting RhoA activity and modulating growth cone dynamics (Lopez-Verrilli et al. 2013). Interestingly, it appears that Schwann cell-derived EVs might be selectively internalized by neurons since they have no noticeable effect on fibroblasts (Lopez-Verrilli et al. 2013). Exosomes from Schwann cells also enhance neurite outgrowth of diabetic DRG neurons challenged with high glucose (Wang et al. 2020). Exosomes derived from other cell types such as undifferentiated adipose derived stem cells (Bucan et al. 2019) and olfactory ensheathing cells also promote DRG neurite outgrowth (Xia et al. 2019). The positive findings from these in vitro studies have been followed up with in vivo studies. In a rat sciatic nerve crush model, injection of adipose stem cell-derived exosomes in the nerve resulted in improved functional recovery (measured by the sciatic function index) and this might be attributed to the uptake of the exosome cargo of neural growth factor transcripts (Bucan et al. 2019). Similarly, bone marrow stem cell-derived exosomes improve outcomes in a crush model and this is correlated with better nerve histomorphology and increases in expression of the pro-regenerative growth associated protein-43 protein (Ma et al. 2017). In more challenging nerve injury models, application of gingival stem cell-derived exosomes in biodegradable chitin nerve conduits (Rao et al. 2019) and olfactory ensheathing cell-derived exosomes in polycaprolactone nerve conduits (Xia et al. 2019) both improved various neurological parameters. Combinatorial approaches, loading cells together with exosomes in conduits, may further enhance the effectiveness of repair, which might be attributed to enhanced cell survival under hypoxic conditions and the increased secretion of neurotrophic growth factors (Zhang et al. 2020). The limitation of these aforementioned in vivo studies is that the neuronal origin of the measured axons is unknown and therefore the regeneration accuracy of these treatments cannot be determined. To specifically address this, a recent study using retrograde labeling examined the effects of EVs in a femoral nerve regeneration model (Madison and Robinson 2019). Injection of denervated muscle-derived EVs enhanced the accuracy of regeneration as measured by the number of motor neurons projecting to the muscle versus the cutaneous branch. Interestingly, in animals that received naïve muscle-derived EV injections, there were no significant differences between the numbers of motor neurons projecting exclusively to the cutaneous or muscle branches. Complementary to this study, in vitro experiments have shown how motor neuron neurite outgrowth and survival is enhanced by muscle cell line-derived EVs (Madison et al. 2014). Primary Schwann cells and Schwann cell-like differentiated adipose stem cells also enhance motor neuron cell line outgrowth (Ching et al. 2018).

Enhancement of primary sensory neuron survival is an important issue to address if clinical outcomes are to be improved after peripheral nerve injury (Hart et al. 2008). To date there are a lack of data on the effects of EVs on DRG neuron survival but results on other culture systems suggest EVs can be neuroprotective. Bone marrow stem cell-derived EVs promote survival of retinal ganglion cells through miRNA-dependent mechanisms (Mead and Tomarev 2017) and priming of the stem cells with TNF- α further enhances their neuroprotective effect (Mead et al. 2020a). Interestingly, fibroblast-derived EVs do not provide any neuroprotection in this

model system (Mead et al. 2020b). In vivo studies indicate that injected bone marrow stem cell-derived exosomes can be transported to the DRG via both retro-grade axonal transport and hematogenous routes, suggesting that EVs could act directly at the soma to influence cell survival (Ren et al. 2019).

4.2 EVs and Schwann Cells

The positive effects of EVs on in vivo axonal regeneration may, in large part, be due to effects on Schwann cells. Adipose stem cell-derived exosomes promote Schwann cell proliferation and migration in vitro (Chen et al. 2019). Exosome-activated Schwann cells display increased neurotrophic factor secretion and enhance myelination of cultured DRG neurons. The EVs are endocytosed preferentially at the Schwann cell processes from where they are transported toward the nucleus (Haertinger et al. 2020). Gingiva stem cell-derived EVs treatment of Schwann cells upregulates the expression of various markers characteristic of the dedifferentiated or repair phenotype, via a mechanism that is dependent on the c-JUN N-terminal kinase pathway (Mao et al. 2019). Exosomes released from differentiated Schwann cells regulate Schwann cell migration through changes in miRNA expression. Next-generation sequencing of exosomes from cAMP-differentiated Schwann cells versus control cells showed numerous nucleic acid species were variably up- or down-regulated (Sohn et al. 2020). The gene targets of those molecules are associated with nerve regeneration and Schwann cell proliferation including tumor necrosis factor pathways, neurotrophin signaling, and perhaps not surprisingly, cAMP-dependent signaling pathways. Despite the well-documented pro-regenerative effects of Schwann cells activated by nerve injury, it has also been reported that the cells may become damaged or undergo apoptosis, particularly under delayed nerve repair conditions (Saito et al. 2009). EVs might therefore be useful for enhancing the survival of the cells. Compared with controls, Schwann cells isolated from injured nerves exhibit lower proliferation rates and increased apoptosis (Liu et al. 2020). These deficits are ameliorated by treatment with adipose stem cell-derived exosomes and the enhanced survival is associated with downregulation of *bax* and increased expression of *bcl-2* genes (Liu et al. 2020). In contrast to the aforementioned studies, it has also been reported that bone marrow stem cell-derived EVs inhibit the proliferation and migration of Schwann cells and enhance their apoptosis via ERK-dependent signaling (Zhou et al. 2018). The reason for these discrepancies might be explained due to the use of primary Schwann cells versus cell lines and the variability in the populations of applied EVs (e.g., exosomes versus microsomes).

4.3 EVs and Neuroimmune Modulation

Schwann cells are not the only important cellular mediators of peripheral nerve regeneration, macrophages also play a key role. Two classes of macrophages can be detected in peripheral nerves, both resident macrophages, which are present under

homeostatic conditions, and infiltrating macrophages which pass into the tissue in response to injury. The resident macrophages, together with neutrophils and Schwann cells, are the first responders after nerve injury. Proliferating resident macrophages begin phagocytosing myelin within days after nerve injury but by the end of the first week post-injury the number of infiltrating macrophages are threefold greater than the resident cells (Mueller et al. 2003). The infiltrating macrophages originate from bone marrow-derived monocytes which are attracted to the injury site in response to the release of monocyte chemoattractive protein produced by dedifferentiating Schwann cells (Subang and Richardson 2001). Collectively, the resident and infiltrating macrophages facilitate the clearance of myelin and axonal debris, which is necessary for the regeneration of axons in the later stages of Wallerian degeneration (Tofaris et al. 2002). The number of macrophages reaches a peak approximately 2 weeks after nerve injury but they remain detectable at 2 months (Taskinen and Roytta 1997).

Although these reactions have positive effects for regeneration, it is critical that they are well controlled since an excessive inflammatory response is associated with neuropathic pain. In rat models, intrathecal injection of exosomes reverses nerve ligation-induced pain (Shiue et al. 2019). The exosomes accumulate in the spinal dorsal horn, DRG, and peripheral axons where they provide anti-inflammatory and pro-neurotrophic functions. Proteins including vascular endothelial growth factor (VEGF)-C, angiopoietin-2, and fibroblast growth factor-2 can be detected in the exosomes. Sensory neuron-macrophage communication in the DRG is altered as a consequence of nerve injury and this may contribute to neuropathic hypersensitivity. The phagocytosis of DRG-derived exosomes, enriched with miR-21, has been proposed as a potential mechanism underlying this (Simeoli et al. 2017). Other studies have indicated critical roles for cargo sorting of vesicular proteins in mediating the signaling mechanisms underlying neuropathic pain (Jean-Toussaint et al. 2020).

Following nerve injury the macrophage populations seen in the ganglia and nerves are heterogeneous with regard to their phenotype and function. There are two classically described polarized phenotypes; M1 macrophages are typically associated with pro-inflammatory functions and degeneration, while M2 macrophages are considered as anti-inflammatory and pro-regenerative. Various studies have suggested that during the early response to nerve injury M1 macrophages predominate but the phenotype shifts with time toward either a non-M1 or an M2 type (Zigmond and Echevarria 2019). M2 macrophage-derived microvesicles enhance the migration and proliferation of Schwann cells and this is associated with elevated expression levels of miR-223 in the M2 EVs (Zhan et al. 2015). The initial infiltrating monocyte reaction could potentially be influenced by the application of EVs. Treatment of monocytes with EVs increases expression of anti-inflammatory molecules such as IL10 and TGF- β 1 and also attenuates the expression of various pro-inflammatory cytokines (Zhang et al. 2014). Furthermore, modulating the phenotype of the infiltrated macrophages with EVs might also boost peripheral nerve regeneration. For instance, this could be done by preconditioning stem cells with LPS to produce EVs with higher expression of anti-inflammatory cytokines which promote the M2 macrophage phenotype (Ti et al. 2015). In summary,

although the inflammatory reaction post-nerve injury is complex and highly orchestrated, it can also be another important target for optimizing peripheral nerve regeneration. Due to the high abundance of over 200 immunomodulatory proteins in MSCs-derived exosomes these, in particular, might be useful adjuncts for nerve repair (Lai et al. 2012).

4.4 EVs and the Neurovascular Reaction

In addition to the aforementioned Schwann cell and immunological reactions, vascular changes around the nerve injury site are important for mediating axon regeneration. It has long ago been observed that ingrowth of blood vessels precedes Schwann cell migration and axon extension (Hobson et al. 1997) and more recently it was shown that macrophages, via the release of VEGF, are responsible for inducing the blood vessels which guide the Schwann cell-mediated regeneration of the axons (Cattin et al. 2015). Vascularization of nerve conduits is necessary to maintain transplanted cell viability and prevent central areas of necrosis which may impede axon regrowth into the distal segment (Muangsanit et al. 2018). Co-transplantation of cells and exosomes in conduits synergistically boosts motor and sensory functions of injured sciatic nerve and this is attributed to the exosome-mediated increase in survival and migration of the transplanted cells under the hypoxic conditions (Zhang et al. 2020). The exosomal cargo mediating these effects was not determined; however, various vasculogenic factors have been described in EVs. Stimulation of angiogenesis by EVs involves transfer of various miRNAs and proteins including VEGF, fibroblast growth factor-2, platelet-derived growth factor (PDGF), and transcription factors STAT3 and STAT5 (Todorova et al. 2017). Vesicles released by endothelial cells play an important role mediating blood vessel formation and this may be associated with their load of active matrix metalloproteinases which promote endothelial cell invasion (Taraboletti et al. 2002). For clinical application, MSC-derived EVs with high angiogenic potential may be a useful adjunct to nerve repair. In vitro conditioning of adipose-derived stem cells increases the angiogenic potential of their secreted EVs. For example, PDGF treatment increases the metalloproteinase content in EVs (Lopatina et al. 2014), and growth of the stem cells in an endothelial differentiation medium increases the pro-angiogenic properties of microvesicles which is associated with increased delivery of miR-31 (Kang et al. 2016). However, to date, the direct role of MSC-derived EVs in modulating vascular regrowth after nerve injury has yet to be confirmed.

5 Characterization of EVs

Despite the rapid growth of pre-clinical research in the field of EVs, the clinical translation of this work is still in its infancy and considerable work needs to be done to develop scalable, reproducible, and GMP-compliant protocols which will fulfill regulatory agency categorization of EVs-based therapeutics. Of utmost importance,

the identity of the therapeutic product needs to be clearly defined. For this, various physicochemical techniques can be used to characterize the EVs, together with biochemical and molecular characterizations of the cargoes of the EVs.

Transmission electron microscopy (TEM) allows sub-nanometer scale resolution of biological structures and can therefore be used to image EVs. Samples are most commonly stained with uranyl acetate or osmium tetroxide but use of cryo-TEM overcomes the need for staining and is therefore better for preserving the structure of the EVs. The morphology of exosomes is described as cup-shaped whereas microvesicles display more heterogeneity in shape. TEM cannot be used to give quantitative measurements so other techniques are required to determine the concentration of EVs. Nanoparticle tracking analysis is a technique that measures particle size distribution and concentration in a liquid suspension, based on the Brownian motion of the particles tracked using laser light scattering (Dragovic et al. 2011). Computer software, incorporating the Stokes–Einstein equation, is used to calculate theoretical diameters of particles recorded by live microscopic imaging. The size distribution of the particles (in other words the EVs) is measurable in the range of 50 nm–1 μ m. Other techniques including flow cytometry, resistive pulse sensing, and more recently Raman spectroscopy have all been described as suitable for the physicochemical characterization of EVs (Gandham et al. 2020).

Since the seminal studies of Valadi and colleagues, which showed intercellular communication could involve the horizontal transfer of RNA via EVs (Valadi et al. 2007), the area has expanded rapidly and nowadays miRNAs are the most extensively studied RNAs found in EVs. The quantity of RNA in EVs can be measured by conventional low volume spectrophotometers such as the NanoDrop™ but electrophoretic-microfluidic based techniques are better for low yield RNA isolates and can be used to determine the relative proportions of different RNA types. qRT-PCR and focused microarrays (e.g., for neural, immune or angiogenic molecules) have been used to identify transcripts relevant to the area of study, and recent reports have described the possibility for next-generation sequencing (RNA-seq) of small RNAs in EVs (Amorim et al. 2017). However, the quality and integrity of the RNA is critical for accurate characterization of the RNA cargo and this may be affected by the method of isolation of the EVs (Buschmann et al. 2018).

Proteins are the other major class of molecule which should be strictly characterized in preparations of EVs. To meet the requirements of the International Society for Extracellular Vesicles, the EVs should express at least one protein from the tetraspanins family (or similar), for example, CD63, CD81, and also a characteristic cytosolic protein such as the heat shock proteins HSP70 and HSP84 (Lotvall et al. 2014). Both total protein quantity and levels of specific proteins can be measured using simple methods such as the Bradford Protein Assay and Western blotting/ELISA, respectively. Although it has been suggested from various studies that specific proteins could be assigned to subpopulations of EVs, this cannot be completely confirmed since the results have been obtained from preparations using different cell types and different isolation procedures. As mentioned previously, if it is necessary to culture cells in serum then it should first be depleted of EVs. It is therefore advisable to measure for albumin as a negative marker to exclude

contamination from supplemented EVs. More extensive proteomic analysis of the EVs, together with metabolomics and lipidomics, enables a deeper identification of the EVs molecular composition.

An extensive characterization of the EVs, used together with predictive potency assays such as neurite outgrowth and neuroprotective and immunomodulatory capacity, can help provide a proposed mode of action for the EVs in the context of nerve repair treatments.

6 Administering EVs

Although a small number of clinical trials have shown that EVs are safe and nontoxic, there is a need for greater understanding of the biodistribution of EVs after their administration. For experimental studies, EVs can be labeled using reporter systems, fluorescent dyes, or superparamagnetic iron oxide nanoparticles and for clinical studies they can be tracked with radioisotope labeling (Chuo et al. 2018). It appears that systemic application of EVs is not ideal since the EVs are rapidly sequestered by circulating macrophages and then accumulate in the liver, spleen, and lungs (Di Rocco et al. 2016). However, the biodistribution may be influenced by the cellular source of the EVs. For example, in a rat model of stroke, it was shown that it is possible for MSC-derived EVs to dose-dependently accumulate in the infarcted brain whereas the transplanted parent cells aggregate in the lungs and liver (Moon et al. 2019). The MSC-EVs which carry the same receptors as their parent cells may thus be recruited to the site of injury via receptor-mediated mechanisms. For optimal targeting it might be necessary to decorate the surface of the EVs with specific proteins which could direct them to specific targets (Antes et al. 2018). The ability to prolong the bioactive lifetime of the EVs at the target site will be critical to their therapeutic efficacy and optimally a sustained delivery system should be used to overcome the rapid clearance of the EVs. The preclinical studies of EVs in peripheral nerve injury have involved application of the EVs via different routes. For nerve crush injury, injection of EVs directly into the nerve was used (Bucan et al. 2019; Ma et al. 2017) and for repair with nerve conduits, EVs have been either added in solution (Rao et al. 2019; Zhang et al. 2020), in a luminal gel filler (Xia et al. 2019), or by intravenous injection (Ma et al. 2019).

The use of biomaterials in nerve repair offers opportunities to optimize the delivery of EVs directly at the injury (Fig. 2). Although there are no reports of direct functionalization of nerve conduits with EVs, studies have shown that EVs combined with engineered scaffolds can contribute to healing and regeneration. MSC-derived EVs have been immobilized on a variety of scaffolds including polylactic-co-glycolic acid (Li et al. 2018) and chitosan/silk sponges (Shi et al. 2017). These materials are frequently used for the fabrication of nerve conduits. For an “off-the-shelf” device it might be possible to freeze-dry the EVs onto the walls of the nerve conduits. Freeze-drying has been suggested to be one of the best methods for maintaining the stability of EVs for long-term storage (Kusuma et al. 2018). Alternatively, the nerve conduit could be filled with a hydrogel containing EVs.

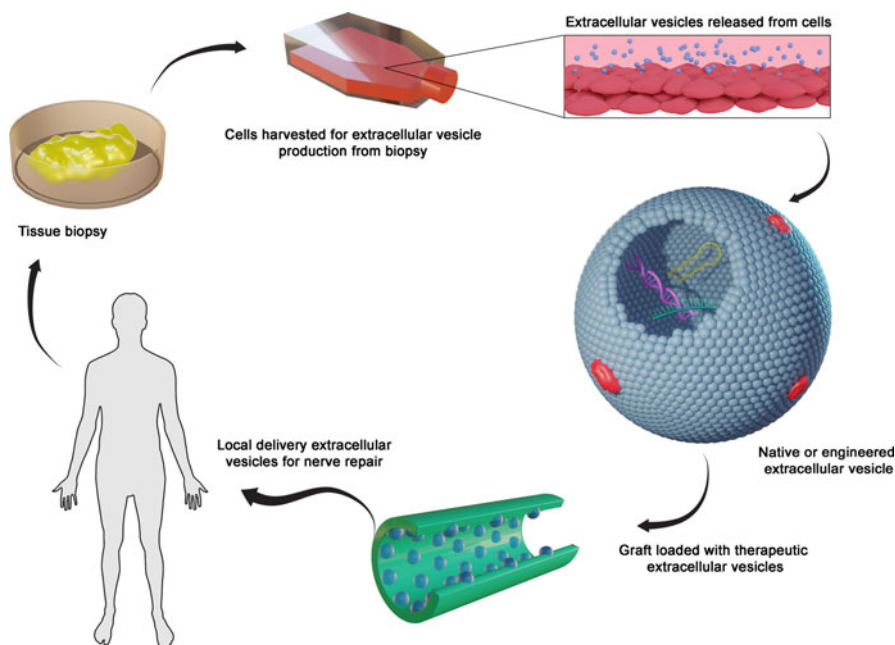


Fig. 2 Extracellular vesicles for a TERM approach to peripheral nerve repair. Various source tissues, for example, adipose tissue, are used for the isolation and expansion of cells needed for production of EVs. The EVs are isolated from cell culture medium using different methods including ultracentrifugation and chromatography, filtration, and precipitation techniques. Native EVs or EVs engineered with specific cargoes or targeting molecules can be incorporated in conduit grafts which are used to reconnect the patient's damaged nerves and facilitate the regeneration of axons

Natural polymers commonly used in nerve repair studies, and for drug delivery, include collagen, alginate, gelatin, and hyaluronic acid. These materials have excellent biocompatibility since they are derived from the native extracellular matrix and importantly they can be customized for controlled degradation rates. Encapsulation of EVs in hydrogels would prevent their premature clearance and facilitate the delivery of a more localized and concentrated dose, directly at the site of nerve injury. Encapsulation can be done by either mixing the EVs with the polymers prior to cross-linking or the EVs can be physically incorporated after the polymerization of the gel in a process known as the “breathing” method (Riau et al. 2019). An outstanding question is what is the most suitable concentration of EVs? The pre-clinical studies show dose-dependent effects of EVs on parameters such as neurite outgrowth but the dose is variably measured by a number of particles or concentration of protein. It is important to note that protein levels cannot be directly correlated with numbers of EVs since there is variable protein abundance depending on the source of EVs and the biogenesis pathways used in their production (Hartjes et al. 2019). The optimal concentration of EVs will likely depend on various factors, such as the cellular source of the EVs, any method used to stimulate the release of the EVs, the makeup of the EVs cargo, and the technique used to deliver the EVs.

7 Engineered EVs

An alternative approach to using native EVs is to engineer them to augment their therapeutic potency. Engineered EVs have been developed as drug delivery agents suitable for transport of various small molecules, nucleic acids, and proteins into cells. Molecules can be loaded into EVs by simple diffusion or through transfection/transduction of the parent cells. Active loading methods, which disrupt the vesicle membranes, include freeze/thawing, sonication, chemical permeabilization, and electroporation (Ramasubramanian et al. 2019). Early studies described the use of small molecule and nucleic acid-engineered EVs in cancer models. For example, the anticancer drug paclitaxel is readily taken up by bone marrow derived MSCs and is then endogenously encapsulated in EVs. Secreted EVs, rich in paclitaxel, can then be harvested from the cell medium and used to inhibit cancer cell growth (Pascucci et al. 2014). More recently, the use of engineered EVs is expanding into the field of regenerative medicine. Silk fibroin hydrogels loaded with EVs enriched with miR-675 (produced by transfection of the parent MSCs) have been used in the treatment of hind limb ischemia (Han et al. 2019). Given the increasing knowledge of miRNAs in neural regenerative medicine (Tang and Sun 2020), the engineering of EVs with selected miRNAs should be a useful additional therapeutic tool. Small interfering RNAs (siRNAs), another class of small RNAs, have also been exogenously loaded into EVs. Exosomal siRNAs have been successfully delivered into neurons where they can mediate anti-apoptotic reactions (de Rivero Vaccari et al. 2016). Loading with exogenous proteins is less well studied and the most effective loading techniques need to be determined. This was partly addressed in a study which showed that the antioxidant enzyme, catalase, could be loaded in exosomes, and impart neuroprotective activity *in vitro* and in a Parkinson's disease model (Haney et al. 2015). In addition to modifying the internal cargo of the EVs, it is possible to modify their surface which can be used for improving targeting of the EVs. Click chemistry has been used to directly, covalently bind molecules to the surface of EVs (Smyth et al. 2014), and membrane proteins such as Lamp2b have been used to fuse specific peptide sequences which enable targeting of neuronal cells (Alvarez-Erviti et al. 2011). Collectively the results of studies with engineered EVs suggest that modifying the internal cargoes or surface markers does not negatively impact on the natural properties of EVs and may therefore be a useful alternative approach to the use of unmanipulated EVs where the active component is poorly defined.

8 Conclusions

Preclinical studies have indicated that EVs promote peripheral nerve regeneration and this may be through direct effects on axon growth, modulation of the Schwann cell phenotype, and interactions with the neuro-immune and vascular reactions occurring as a consequence of nerve injury. The bioactivity of the EVs is mediated by their cargo which can contain various neuroregenerative nucleic acids and proteins. Since EVs, in large part, replicate the regenerative functions of their parent

cells, they represent an alternative therapeutic approach to that of cell transplantation which is associated with poor cell engraftment and low viability and limited by cell immunogenicity. EVs can thus clearly become an important component of TERM approaches for peripheral nerve repair. Nevertheless, challenges remain. In particular, scalable and reproducible protocols for the production and isolation of high yields of EVs are needed. A standardized manufacturing process for EVs, in compliance with GMP, is necessary if EVs are to become mainstream clinical therapies.

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References

- Akers JC, Gonda D, Kim R, Carter BS, Chen CC (2013) Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. *J Neuro-Oncol* 113:1–11. <https://doi.org/10.1007/s11060-013-1084-8>
- Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ (2011) Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol* 29:341–345. <https://doi.org/10.1038/nbt.1807>
- Amorim MG et al (2017) A total transcriptome profiling method for plasma-derived extracellular vesicles: applications for liquid biopsies. *Sci Rep* 7:14395. <https://doi.org/10.1038/s41598-017-14264-5>
- Antes TJ et al (2018) Targeting extracellular vesicles to injured tissue using membrane cloaking and surface display. *J Nanobiotechnology* 16:61. <https://doi.org/10.1186/s12951-018-0388-4>
- Ashraf R, Sofi HS, Beigh MA, Sheikh FA (2019) Recent trends in peripheral nervous regeneration using 3D biomaterials. *Tissue Cell* 59:70–81. <https://doi.org/10.1016/j.tice.2019.06.003>
- Bjorge IM, Kim SY, Mano JF, Kalionis B, Chrzanowski W (2017) Extracellular vesicles, exosomes and shedding vesicles in regenerative medicine – a new paradigm for tissue repair. *Biomater Sci* 6:60–78. <https://doi.org/10.1039/c7bm00479f>
- Brini AT et al (2017) Therapeutic effect of human adipose-derived stem cells and their secretome in experimental diabetic pain. *Sci Rep* 7:9904. <https://doi.org/10.1038/s41598-017-09487-5>
- Bucan V, Vaslaitis D, Peck CT, Strauss S, Vogt PM, Radtke C (2019) Effect of exosomes from rat adipose-derived mesenchymal stem cells on neurite outgrowth and sciatic nerve regeneration after crush injury. *Mol Neurobiol* 56:1812–1824. <https://doi.org/10.1007/s12035-018-1172-z>
- Buschmann D et al (2018) Evaluation of serum extracellular vesicle isolation methods for profiling miRNAs by next-generation sequencing. *J Extracell Vesicles* 7:1481321. <https://doi.org/10.1080/20013078.2018.1481321>
- Carvalho CR, Oliveira JM, Reis RL (2019) Modern trends for peripheral nerve repair and regeneration: beyond the hollow nerve guidance conduit. *Front Bioeng Biotechnol* 7:337. <https://doi.org/10.3389/fbioe.2019.00337>
- Cattin AL et al (2015) Macrophage-induced blood vessels guide Schwann cell-mediated regeneration of peripheral nerves. *Cell* 162:1127–1139. <https://doi.org/10.1016/j.cell.2015.07.021>
- Chen J et al (2019) Exosomes from human adipose-derived stem cells promote sciatic nerve regeneration via optimizing Schwann cell function. *J Cell Physiol* 234:23097–23110. <https://doi.org/10.1002/jcp.28873>

- Ching RC, Wiberg M, Kingham PJ (2018) Schwann cell-like differentiated adipose stem cells promote neurite outgrowth via secreted exosomes and RNA transfer. *Stem Cell Res Ther* 9:266. <https://doi.org/10.1186/s13287-018-1017-8>
- Chuo ST, Chien JC, Lai CP (2018) Imaging extracellular vesicles: current and emerging methods. *J Biomed Sci* 25:91. <https://doi.org/10.1186/s12929-018-0494-5>
- Crescitelli R et al (2013) Distinct RNA profiles in subpopulations of extracellular vesicles: apoptotic bodies, microvesicles and exosomes. *J Extracell Vesicles* 2. <https://doi.org/10.3402/jev.v2i0.20677>
- de Rivero Vaccari JP et al (2016) Exosome-mediated inflammasome signaling after central nervous system injury. *J Neurochem* 136(Suppl 1):39–48. <https://doi.org/10.1111/jnc.13036>
- Di Rocco G, Baldari S, Toietta G (2016) Towards therapeutic delivery of extracellular vesicles: strategies for in vivo tracking and biodistribution analysis. *Stem Cells Int* 2016:5029619. <https://doi.org/10.1155/2016/5029619>
- Dong R, Liu Y, Yang Y, Wang H, Xu Y, Zhang Z (2019) MSC-Derived exosomes-based therapy for peripheral nerve injury: a novel therapeutic strategy. *Biomed Res Int* 2019:6458237. <https://doi.org/10.1155/2019/6458237>
- Dragovic RA et al (2011) Sizing and phenotyping of cellular vesicles using nanoparticle tracking analysis. *Nanomedicine* 7:780–788. <https://doi.org/10.1016/j.nano.2011.04.003>
- Eldh M, Ekstrom K, Valadi H, Sjostrand M, Olsson B, Jernas M, Lotvall J (2010) Exosomes communicate protective messages during oxidative stress; possible role of exosomal shuttle RNA. *PLoS One* 5:e15353. <https://doi.org/10.1371/journal.pone.0015353>
- Ezquer FE, Ezquer ME, Vicencio JM, Calligaris SD (2017) Two complementary strategies to improve cell engraftment in mesenchymal stem cell-based therapy: increasing transplanted cell resistance and increasing tissue receptivity. *Cell Adhes Migr* 11:110–119. <https://doi.org/10.1080/19336918.2016.1197480>
- Feng D et al (2010) Cellular internalization of exosomes occurs through phagocytosis. *Traffic* 11:675–687. <https://doi.org/10.1111/j.1600-0854.2010.01041.x>
- Gama KB et al (2018) Conditioned medium of bone marrow-derived mesenchymal stromal cells as a therapeutic approach to neuropathic pain: a preclinical evaluation. *Stem Cells Int* 2018:8179013. <https://doi.org/10.1155/2018/8179013>
- Gandham S et al (2020) Technologies and standardization in research on extracellular vesicles. *Trends Biotechnol*. <https://doi.org/10.1016/j.tibtech.2020.05.012>
- Gibbins DJ, Ciaudo C, Erhardt M, Voinnet O (2009) Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. *Nat Cell Biol* 11:1143–1149. <https://doi.org/10.1038/ncb1929>
- Grinblat GA, Khan RS, Dine K, Wessel H, Brown L, Shindler KS (2018) RGC Neuroprotection Following Optic Nerve Trauma Mediated By Intranasal Delivery of Amnion Cell Secretome. *Invest Ophthalmol Vis Sci* 59:2470–2477. <https://doi.org/10.1167/iovs.18-24096>
- Guo SC, Tao SC, Dawn H (2018) Microfluidics-based on-a-chip systems for isolating and analysing extracellular vesicles. *J Extracell Vesicles* 7:1508271. <https://doi.org/10.1080/20013078.2018.1508271>
- Gutmann E (1962) Denervation and disuse atrophy in crossstriated muscle. *Rev Can Biol* 21:353–365
- Haertinger M, Weiss T, Mann A, Tabi A, Brandel V, Radtke C (2020) Adipose stem cell-derived extracellular vesicles induce proliferation of schwann cells via internalization. *Cell* 9:163. <https://doi.org/10.3390/cells9010163>
- Han C et al (2019) Delivery of miR-675 by stem cell-derived exosomes encapsulated in silk fibroin hydrogel prevents aging-induced vascular dysfunction in mouse hindlimb. *Mater Sci Eng C Mater Biol Appl* 99:322–332. <https://doi.org/10.1016/j.msec.2019.01.122>
- Haney MJ et al (2015) Exosomes as drug delivery vehicles for Parkinson's disease therapy. *J Control Release* 207:18–30. <https://doi.org/10.1016/j.jconrel.2015.03.033>
- Hart AM, Terenghi G, Wiberg M (2008) Neuronal death after peripheral nerve injury and experimental strategies for neuroprotection. *Neurol Res* 30:999–1011. <https://doi.org/10.1179/174313208X362479>

- Hartjes TA, Mytnyk S, Jenster GW, van Steijn V, van Royen ME (2019) Extracellular vesicle quantification and characterization: common methods and emerging approaches. *Bioengineering* (Basel):6. <https://doi.org/10.3390/bioengineering6010007>
- Helwa I et al (2017) A comparative study of serum exosome isolation using differential ultracentrifugation and three commercial reagents. *PLoS One* 12:e0170628. <https://doi.org/10.1371/journal.pone.0170628>
- Hobson MI, Brown R, Green CJ, Terenghi G (1997) Inter-relationships between angiogenesis and nerve regeneration: a histochemical study. *Br J Plast Surg* 50:125–131. [https://doi.org/10.1016/s0007-1226\(97\)91325-4](https://doi.org/10.1016/s0007-1226(97)91325-4)
- Jangamreddy JR et al (2018) Short peptide analogs as alternatives to collagen in pro-regenerative corneal implants. *Acta Biomater* 69:120–130. <https://doi.org/10.1016/j.actbio.2018.01.011>
- Jean-Toussaint R, Tian Y, Chaudhuri AD, Haughey NJ, Sacan A, Ajit SK (2020) Proteome characterization of small extracellular vesicles from spared nerve injury model of neuropathic pain. *J Proteome* 211:103540. <https://doi.org/10.1016/j.jprot.2019.103540>
- Kang T et al (2016) Adipose-derived stem cells induce angiogenesis via microvesicle transport of miRNA-31. *Stem Cells Transl Med* 5:440–450. <https://doi.org/10.5966/sctm.2015-0177>
- Kehoe S, Zhang XF, Boyd D (2012) FDA approved guidance conduits and wraps for peripheral nerve injury: a review of materials and efficacy. *Injury* 43:553–572. <https://doi.org/10.1016/j.injury.2010.12.030>
- Kolar MK, Kingham PJ (2014) Regenerative effects of adipose-tissue-derived stem cells for treatment of peripheral nerve injuries. *Biochem Soc Trans* 42:697–701. <https://doi.org/10.1042/BST20140004>
- Krawczyński A, Bielawska-Pohl A, Paprocka M, Kraskiewicz H, Szyposzynska A, Wojdat E, Klimczak A (2020) Microvesicles from human immortalized cell lines of endothelial progenitor cells and mesenchymal stem/stromal cells of adipose tissue origin as carriers of bioactive factors facilitating angiogenesis. *Stem Cells Int* 2020:1289380. <https://doi.org/10.1155/2020/1289380>
- Kusuma GD, Barabadi M, Tan JL, Morton DAV, Frith JE, Lim R (2018) To protect and to preserve: novel preservation strategies for extracellular vesicles. *Front Pharmacol* 9:1199. <https://doi.org/10.3389/fphar.2018.01199>
- Lai RC et al (2012) Proteolytic potential of the msc exosome proteome: implications for an exosome-mediated delivery of therapeutic proteasome. *Int J Proteomics* 2012:971907. <https://doi.org/10.1155/2012/971907>
- Li W et al (2018) Tissue-engineered bone immobilized with human adipose stem cells-derived exosomes promotes bone regeneration. *ACS Appl Mater Interfaces* 10:5240–5254. <https://doi.org/10.1021/acsami.7b17620>
- Liu CY et al (2020) Effect of exosomes from adipose-derived stem cells on the apoptosis of Schwann cells in peripheral nerve injury. *CNS Neurosci Ther* 26:189–196. <https://doi.org/10.1111/cns.13187>
- Lopatina T, Bruno S, Tetta C, Kalinina N, Porta M, Camussi G (2014) Platelet-derived growth factor regulates the secretion of extracellular vesicles by adipose mesenchymal stem cells and enhances their angiogenic potential. *Cell Commun Signal* 12:26. <https://doi.org/10.1186/1478-811X-12-26>
- Lopez-Verrilli MA, Picou F, Court FA (2013) Schwann cell-derived exosomes enhance axonal regeneration in the peripheral nervous system. *Glia* 61:1795–1806. <https://doi.org/10.1002/glia.22558>
- Lotvall J et al (2014) Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. *J Extracell Vesicles* 3:26913. <https://doi.org/10.3402/jev.v3.26913>
- Ma Y, Ge S, Zhang J, Zhou D, Li L, Wang X, Su J (2017) Mesenchymal stem cell-derived extracellular vesicles promote nerve regeneration after sciatic nerve crush injury in rats. *Int J Clin Exp Pathol* 10:10032–10039

- Ma Y et al (2019) Extracellular vesicles from human umbilical cord mesenchymal stem cells improve nerve regeneration after sciatic nerve transection in rats. *J Cell Mol Med* 23:2822–2835. <https://doi.org/10.1111/jcmm.14190>
- Madison RD, Robinson GA (2019) Muscle-derived extracellular vesicles influence motor neuron regeneration accuracy. *Neuroscience* 419:46–59. <https://doi.org/10.1016/j.neuroscience.2019.08.028>
- Madison RD, McGee C, Rawson R, Robinson GA (2014) Extracellular vesicles from a muscle cell line (C2C12) enhance cell survival and neurite outgrowth of a motor neuron cell line (NSC-34). *J Extracell Vesicles* 3. <https://doi.org/10.3402/jev.v3.22865>
- Mao Q, Nguyen PD, Shanti RM, Shi S, Shakoori P, Zhang Q, Le AD (2019) Gingiva-derived mesenchymal stem cell-extracellular vesicles activate schwann cell repair phenotype and promote nerve regeneration tissue. *Eng Part A* 25:887–900. <https://doi.org/10.1089/ten.TEA.2018.0176>
- Mead B, Tomarev S (2017) Bone marrow-derived mesenchymal stem cells-derived exosomes promote survival of retinal ganglion cells through mirna-dependent mechanisms. *Stem Cells Transl Med* 6:1273–1285. <https://doi.org/10.1002/sctm.16-0428>
- Mead B, Chamling X, Zack DJ, Ahmed Z, Tomarev S (2020a) TNF α -mediated priming of mesenchymal stem cells enhances their neuroprotective effect on retinal ganglion cells. *Invest Ophthalmol Vis Sci* 61:6. <https://doi.org/10.1167/iops.61.2.6>
- Mead B, Cullather E, Nakaya N, Niu Y, Kole C, Ahmed Z, Tomarev S (2020b) Viral delivery of multiple miRNAs promotes retinal ganglion cell survival and functional preservation after optic nerve crush injury. *Exp Eye Res* 197:108071. <https://doi.org/10.1016/j.exer.2020.108071>
- Moon GJ et al (2019) Application of mesenchymal stem cell-derived extracellular vesicles for stroke: biodistribution and microrna study. *Transl Stroke Res* 10:509–521. <https://doi.org/10.1007/s12975-018-0668-1>
- Morelli AE et al (2004) Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. *Blood* 104:3257–3266. <https://doi.org/10.1182/blood-2004-03-0824>
- Muangsanit P, Shipley RJ, Phillips JB (2018) Vascularization strategies for peripheral nerve tissue engineering. *Anat Rec (Hoboken)* 301:1657–1667. <https://doi.org/10.1002/ar.23919>
- Mueller M, Leonhard C, Wacker K, Ringelstein EB, Okabe M, Hickey WF, Kiefer R (2003) Macrophage response to peripheral nerve injury: the quantitative contribution of resident and hematogenous macrophages. *Lab Invest* 83:175–185. <https://doi.org/10.1097/01.lab.0000056993.28149.bf>
- Mulcahy LA, Pink RC, Carter DR (2014) Routes and mechanisms of extracellular vesicle uptake. *J Extracell Vesicles* 3. <https://doi.org/10.3402/jev.v3.24641>
- Nanbo A, Kawanishi E, Yoshida R, Yoshiyama H (2013) Exosomes derived from Epstein-Barr virus-infected cells are internalized via caveola-dependent endocytosis and promote phenotypic modulation in target cells. *J Virol* 87:10334–10347. <https://doi.org/10.1128/JVI.01310-13>
- Pascucci L et al (2014) Paclitaxel is incorporated by mesenchymal stromal cells and released in exosomes that inhibit in vitro tumor growth: a new approach for drug delivery. *J Control Release* 192:262–270. <https://doi.org/10.1016/j.jconrel.2014.07.042>
- Patel DB, Gray KM, Santharam Y, Lamichhane TN, Stroka KM, Jay SM (2017) Impact of cell culture parameters on production and vascularization bioactivity of mesenchymal stem cell-derived extracellular vesicles. *Bioeng Transl Med* 2:170–179. <https://doi.org/10.1002/btm2.10065>
- Patel DB, Santoro M, Born LJ, Fisher JP, Jay SM (2018) Towards rationally designed biomanufacturing of therapeutic extracellular vesicles: impact of the bioproduction microenvironment. *Biotechnol Adv* 36:2051–2059. <https://doi.org/10.1016/j.biotechadv.2018.09.001>
- Ramasubramanian L, Kumar P, Wang A (2019) Engineering extracellular vesicles as nano-therapeutics for regenerative medicine. *Biomolecules* 10. <https://doi.org/10.3390/biom10010048>

- Rao F et al (2019) Exosomes from human gingiva-derived mesenchymal stem cells combined with biodegradable chitin conduits promote rat sciatic nerve regeneration. *Stem Cells Int* 2019:2546367. <https://doi.org/10.1155/2019/2546367>
- Ren R et al (2019) Bone marrow mesenchymal stem cell-derived exosome uptake and retrograde transport can occur at peripheral nerve endings. *Artif Cells Nanomed Biotechnol* 47:2918–2929. <https://doi.org/10.1080/21691401.2019.1640713>
- Riau AK, Ong HS, Yam GHF, Mehta JS (2019) Sustained delivery system for stem cell-derived exosomes. *Front Pharmacol* 10:1368. <https://doi.org/10.3389/fphar.2019.01368>
- Rotshenker S (2011) Wallerian degeneration: the innate-immune response to traumatic nerve injury. *J Neuroinflammation* 8:109. <https://doi.org/10.1186/1742-2094-8-109>
- Saito H, Kanje M, Dahlin LB (2009) Delayed nerve repair increases number of caspase 3 stained Schwann cells. *Neurosci Lett* 456:30–33. <https://doi.org/10.1016/j.neulet.2009.03.075>
- Shi Q et al (2017) GMSC-derived exosomes combined with a chitosan/silk hydrogel sponge accelerates wound healing in a diabetic rat skin defect model. *Front Physiol* 8:904. <https://doi.org/10.3389/fphys.2017.00904>
- Shiue SJ, Rau RH, Shiue HS, Hung YW, Li ZX, Yang KD, Cheng JK (2019) Mesenchymal stem cell exosomes as a cell-free therapy for nerve injury-induced pain in rats. *Pain* 160:210–223. <https://doi.org/10.1097/j.pain.0000000000001395>
- Siemionow M, Brzezicki G (2009) Chapter 8: Current techniques and concepts in peripheral nerve repair. *Int Rev Neurobiol* 87:141–172. [https://doi.org/10.1016/S0074-7742\(09\)87008-6](https://doi.org/10.1016/S0074-7742(09)87008-6)
- Simeoli R et al (2017) Exosomal cargo including microRNA regulates sensory neuron to macrophage communication after nerve trauma. *Nat Commun* 8:1778. <https://doi.org/10.1038/s41467-017-01841-5>
- Smyth T et al (2014) Surface functionalization of exosomes using click chemistry. *Bioconjug Chem* 25:1777–1784. <https://doi.org/10.1021/bc500291r>
- Sohn EJ, Park HT, Shin YK (2020) Exosomes derived from differentiated Schwann cells inhibit Schwann cell migration via microRNAs. *Neuroreport* 31:515–522. <https://doi.org/10.1097/WNR.0000000000001435>
- Stephens L, Ellison C, Hawkins P (2002) Roles of PI3Ks in leukocyte chemotaxis and phagocytosis. *Curr Opin Cell Biol* 14:203–213. [https://doi.org/10.1016/s0955-0674\(02\)00311-3](https://doi.org/10.1016/s0955-0674(02)00311-3)
- Subang MC, Richardson PM (2001) Influence of injury and cytokines on synthesis of monocyte chemoattractant protein-1 mRNA in peripheral nervous tissue. *Eur J Neurosci* 13:521–528. <https://doi.org/10.1046/j.1460-9568.2001.01425.x>
- Sugimura-Wakayama Y, Katagiri W, Osugi M, Kawai T, Ogata K, Sakaguchi K, Hibi H (2015) Peripheral nerve regeneration by secretomes of stem cells from human exfoliated deciduous teeth. *Stem Cells Dev* 24:2687–2699. <https://doi.org/10.1089/scd.2015.0104>
- Tang X, Sun C (2020) The roles of MicroRNAs in neural regenerative medicine. *Exp Neurol* 332:113394. <https://doi.org/10.1016/j.expneurol.2020.113394>
- Tapp M, Wenzinger E, Tarabishy S, Ricci J, Herrera FA (2019) The epidemiology of upper extremity nerve injuries and associated cost in the US emergency departments. *Ann Plast Surg* 83:676–680. <https://doi.org/10.1097/SAP.0000000000002083>
- Taraboletti G, D'Ascenzo S, Borsotti P, Giavazzi R, Pavan A, Dolo V (2002) Shedding of the matrix metalloproteinases MMP-2, MMP-9, and MT1-MMP as membrane vesicle-associated components by endothelial cells. *Am J Pathol* 160:673–680. [https://doi.org/10.1016/S0002-9440\(10\)64887-0](https://doi.org/10.1016/S0002-9440(10)64887-0)
- Taskinen HS, Roytta M (1997) The dynamics of macrophage recruitment after nerve transection. *Acta Neuropathol* 93:252–259. <https://doi.org/10.1007/s004010050611>
- Thery C (2011) Exosomes: secreted vesicles and intercellular communications F1000. *Biol Reprod* 3:15. <https://doi.org/10.3410/B3-15>
- Thery C et al (2018) Minimal information for studies of extracellular vesicles 2018 (MISEV 2018): a position statement of the international society for extracellular vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* 7:1535750. <https://doi.org/10.1080/20013078.2018.1535750>

- Ti D et al (2015) LPS-preconditioned mesenchymal stromal cells modify macrophage polarization for resolution of chronic inflammation via exosome-shuttled let-7b. *J Transl Med* 13:308. <https://doi.org/10.1186/s12967-015-0642-6>
- Todorova D, Simoncini S, Lacroix R, Sabatier F, Dignat-George F (2017) Extracellular vesicles in angiogenesis. *Circ Res* 120:1658–1673. <https://doi.org/10.1161/CIRCRESAHA.117.309681>
- Tofaris GK, Patterson PH, Jessen KR, Mirsky R (2002) Denervated Schwann cells attract macrophages by secretion of leukemia inhibitory factor (LIF) and monocyte chemoattractant protein-1 in a process regulated by interleukin-6 and LIF. *J Neurosci* 22:6696–6703. <https://doi.org/10.1523/JNEUROSCI.22-15-06696.2002>
- Tohill M, Mantovani C, Wiberg M, Terenghi G (2004) Rat bone marrow mesenchymal stem cells express glial markers and stimulate nerve regeneration. *Neurosci Lett* 362:200–203. <https://doi.org/10.1016/j.neulet.2004.03.077>
- Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO (2007) Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 9:654–659. <https://doi.org/10.1038/ncb1596>
- Wakao S, Matsuse D, Dezawa M (2014) Mesenchymal stem cells as a source of Schwann cells: their anticipated use in peripheral nerve regeneration. *Cells Tissues Organs* 200:31–41. <https://doi.org/10.1159/000368188>
- Wang LH, Rothberg KG, Anderson RG (1993) Mis-assembly of clathrin lattices on endosomes reveals a regulatory switch for coated pit formation. *J Cell Biol* 123:1107–1117. <https://doi.org/10.1083/jcb.123.5.1107>
- Wang L et al (2020) Exosomes derived from Schwann cells ameliorate peripheral neuropathy in type 2 diabetic mice. *Diabetes* 69:749–759. <https://doi.org/10.2337/db19-0432>
- Watson DC et al (2016) Efficient production and enhanced tumor delivery of engineered extracellular vesicles. *Biomaterials* 105:195–205. <https://doi.org/10.1016/j.biomaterials.2016.07.003>
- Watson DC et al (2018) Scalable, cGMP-compatible purification of extracellular vesicles carrying bioactive human heterodimeric IL-15/lactadherin complexes. *J Extracell vesicles* 7:1442088. <https://doi.org/10.1080/20013078.2018.1442088>
- West CA, Hart AM, Terenghi G, Wiberg M (2007) Analysis of the dose-response of N-acetylcysteine in the prevention of sensory neuronal loss after peripheral nerve injury. *Acta Neurochir Suppl* 100:29–31. https://doi.org/10.1007/978-3-211-72958-8_6
- Xia B et al (2019) Extracellular vesicles derived from olfactory ensheathing cells promote peripheral nerve regeneration in rats. *Front Cell Neurosci* 13:548. <https://doi.org/10.3389/fncel.2019.00548>
- Zhan C, Ma CB, Yuan HM, Cao BY, Zhu JJ (2015) Macrophage-derived microvesicles promote proliferation and migration of Schwann cell on peripheral nerve repair. *Biochem Biophys Res Commun* 468:343–348. <https://doi.org/10.1016/j.bbrc.2015.10.097>
- Zhang B, Yin Y, Lai RC, Tan SS, Choo AB, Lim SK (2014) Mesenchymal stem cells secrete immunologically active exosomes. *Stem Cells Dev* 23:1233–1244. <https://doi.org/10.1089/scd.2013.0479>
- Zhang H et al (2018) Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric flow field-flow fractionation. *Nat Cell Biol* 20:332–343. <https://doi.org/10.1038/s41556-018-0040-4>
- Zhang Y, Wang WT, Gong CR, Li C, Shi M (2020) Combination of olfactory ensheathing cells and human umbilical cord mesenchymal stem cell-derived exosomes promotes sciatic nerve regeneration. *Neural Regen Res* 15:1903–1911. <https://doi.org/10.4103/1673-5374.280330>
- Zhou D, Zhai W, Zhang M (2018) Mesenchymal stem cell-derived extracellular vesicles promote apoptosis in RSC96 Schwann cells through the activation of the ERK pathway. *Int J Clin Exp Pathol* 11:5157–5170
- Zigmond RE, Echevarria FD (2019) Macrophage biology in the peripheral nervous system after injury. *Prog Neurobiol* 173:102–121. <https://doi.org/10.1016/j.pneurobio.2018.12.001>

Drug Therapies for Peripheral Nerve Injuries

Melissa L. D. Rayner, Jess Healy, and James B. Phillips

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Abstract

Following a peripheral nerve injury (PNI), neurons have the capacity to regenerate but the rate is remarkably slow. Microsurgical treatments are used to reconnect the nerve stumps following a PNI and encourage the regeneration of axons; however, there are currently no therapies available to promote the rate of this regeneration. Drug therapies available for PNI tend to focus on the resulting symptoms such as neuropathic pain, inflammation, and weakness without modifying the condition itself. Effectively designed pharmacological treatments could potentially increase the regenerative rate as well as maintain neuronal viability and improve axonal specificity to target organs. Appropriate drug agents need to target specific events following a nerve injury and advancements in understanding of the molecular and cellular cascades which follow injury could inform this. Some drugs and targets within signaling pathways have been identified, but challenges remain with clinical translation.

1 Introduction

Peripheral nerve injury (PNI) has a high prevalence affecting all patient populations (Chan et al. 2014) and the associated nerve damage can be debilitating, resulting in life-long loss or disturbance in end-organ function, which compromises the patient's quality of life (Fex Svehnigsen and Dahlin 2013). PNI can be attributed to diseases, such as diabetic neuropathy, and drug therapies such as chemotherapy or radiotherapy (Jones et al. 2016). However, the most common cause of PNI is trauma, much of which results from motor vehicle accidents, surgery, and births (Jones et al. 2016).

The principal clinical treatment for PNI has not changed in over 30 years despite an improved understanding of the associated neuropathophysiology (Faroni et al. 2015). Current treatments employ surgical intervention with a primary repair between the proximal and distal stump remaining the gold standard for a transection injury with no tension, and an autograft for long gap repair (Faroni et al. 2015). Surgical treatments fail to address the complexity of the events that occur following a PNI (Faroni et al. 2015), and adequate functional improvement is not always achieved. Therefore, there is a clear clinical need to find new approaches, such as drug treatments, that could complement existing surgical approaches and improve functional recovery.

Unlike the central nervous system, the peripheral nervous system has some capacity to regenerate naturally (Hoke and Brushart 2010) with a rate of ~1 mm per day; however, this can vary widely from 0.5 to 9 mm per day (Burnett and Zager 2004; Chan et al. 2014). Successful regeneration is highly dependent on both efficient axon regrowth and myelination of those axons by Schwann cells (Chen et al. 2007) (see chapter ► “Schwann Cells in Nerve Repair and Regeneration”). Delay in regeneration results in the loss of Schwann cells in the distal stump, which in turn will cause them to lose their supportive role in guiding axon growth (Poppler et al. 2016). Furthermore, a lack of connection between the axons and target organ results in atrophy of muscles and associated loss of functional recovery (Dubovy et al. 2013; Fu and Gordon 1995; Hoke and Brushart 2010; Isaacs 2013).

2 Potential Cellular Targets for Drug Therapy

The capacity for functional regeneration is directly dependent upon the type of injury, degree of axonal guidance, and the proximity of the injury to the nerve cell body (Faroni et al. 2015). Following a peripheral nerve crush or axotomy, it is evident that many molecular and cellular changes occur at three main sites: the neuronal cell body, site of injury, and end-organ target. Within these sites, three key cell types have been identified as potential targets for improving regeneration namely: neurons, Schwann cells, and macrophages (Gomez-Sanchez et al. 2015; Mokarram et al. 2012). Enhanced understanding of the role that each of these cell types plays in regeneration will aid the identification of potential pharmacological targets.

2.1 Neurons

Large numbers of neurons are at risk of death following PNI; however, quantification of this loss is challenging and highly dependent on the experimental model and analytical method for quantification used (Hart et al. 2008). Neuron cell survival is vital for initial axon sprouting within the proximal segment, the subsequent regeneration to the distal segment, and ultimately reinnervation of the end-organ. The withdrawal of target-derived neurotrophic factors determines gene and protein expression for either regeneration or cell apoptosis. Following an injury, both the regenerative and cell death pathways are triggered and the cell's fate depends highly on the balance of these two opposing pathways (Nunez and Del Peso 1998). Neuronal cell death is relatively slow (15–20 h), which provides an opportunistic time-frame for pharmacological intervention to provide neuro-protection and promote regeneration (Faroni et al. 2015). Furthermore, following PNI the cell body receives a signal of a high frequency burst of action potentials which causes the opening of calcium channels and subsequent initiation of the Jun-kinase cascade which in turn influences transcription (Faroni et al. 2015). This results in an upregulation of specific axonal proteins, such as GAP43, and an upregulation in the expression of regeneration-associated genes (RAGs) which act to promote regeneration (Ma and Willis 2015; Schmitt et al. 2003).

Nimodipine, a calcium channel blocker, has shown to improve regeneration by reducing calcium-induced depolarization in these growth cones. The improvement was due to an increase in the calcium-binding S-100 β protein and less inflammation (Mattsson et al. 2001; Tang et al. 2015). In a rat facial nerve injury model, there was evidence of re-myelination alongside nerve regeneration (Tang et al. 2015). Nimodipine has also been tested in 2 clinical studies which both resulted in benefits in functional recovery. In the first study, 13 patients received a daily dose of 360 mg nimodipine following facial nerve damage as a result of maxillofacial surgery. The patients demonstrated functional recovery within 2 months of treatment (Scheller and Scheller 2012). In the second study, 19 patients received 30 mg twice daily following a direct repair or graft on a unilateral transection in the laryngeal nerve, which was associated with improvements in reinnervation (Mattsson et al. 2018).

Despite the positive results, significant limitations were present in both studies due to a low sample size and no control group.

2.2 Schwann Cells

Schwann cells are key facilitators in nerve regeneration and exert control over molecular mechanisms and pathways to aid development, myelination, and myelin maintenance (Jessen et al. 2008). Hall (1986) demonstrated the vital role Schwann cells play in the regeneration process using a mouse sciatic nerve gap (0.5 mm) model repaired with an autograft. A reduction in the quality and extent of regeneration in the absence of Schwann cells was shown (Hall 1986).

Schwann cells have the ability to reprogram between phenotypes. Cells switch to a repair (Büngner) cell phenotype shortly after injury, thus switching from a role in maintenance of axonal ensheathment to that of regeneration. Following Wallerian degeneration, the Schwann cells adopt an elongated bipolar morphology and form bands of Büngner which act as a conduit to guide the regeneration of the axon (Deumens et al. 2010; Gaudet et al. 2011; Hall 2005; Jessen and Mirsky 2016).

In the proximal segment, Schwann cells provide vital support for regenerating axons; however, in the chronically denervated distal segment, Schwann cells degenerate and can enter a senescent state. This means they undergo irreversible growth arrest and can no longer play a supportive role to the regenerating axon. The exact mechanism that drives Schwann cells into senescence is unknown; however, it has been suggested that longer nerve defects place a higher demand on Schwann cell proliferation which leads to senescence (Saheb-Al-Zamani et al. 2013). Preventing Schwann cell senescence is, therefore, an attractive potential target for pharmacological treatments in PNI. Resveratrol, an activator of the sirtuin enzymes (mainly SIRT1), has demonstrated the ability to block/prevent cellular senescence (Demidenko and Blagosklonny 2009; Matos et al. 2017; Stefani et al. 2007; Yuan et al. 2013). In addition, when resveratrol treatment was combined with transplanted Schwann cells in a rat sciatic nerve crush injury, the expression levels of growth factors increased and improved regeneration and function were observed (Elbaz et al. 2017).

2.3 Macrophages

The primary role of macrophages in peripheral nerve regeneration is demyelination and cellular debris clear up at the injury site and within the degenerating distal segment during Wallerian degeneration (Hall 2005). Macrophages present a spectrum of phenotypes which can be classified broadly into two groups: pro-inflammatory M1 cells and pro-regenerative M2 cells (Mokarram et al. 2012; Martinez and Gordon 2014). M2 macrophage cells result in an increased rate of Schwann cell migration relative to cells in the M1 phenotype. The pro-regenerative M2 cells are split further into three subphenotypes: M2a, M2b, and M2c. M2a and M2c act to enhance tissue repair and promote healing, whereas M2b plays a regulatory role (Mokarram et al. 2012).

There are two sources of macrophages during peripheral nervous system injury: resident and recruited. The resident macrophages account for 4% of the endoneural cellular population and respond rapidly to injury. Recruited macrophages are attracted to the site of injury by chemokines expressed by denervated Schwann cells. These recruited macrophages promote regeneration by secreting apolipoprotein E (Apo E) as they scavenge lipids to deliver to Schwann cells for re-use in axon re-growth (Hall 2005). They also act as a source of axonal regeneration-related factors, such as extracellular matrix (ECM), growth factors, cytokines, and chemokines, and promote vascularization in the distal stump (Jessen and Mirsky 2016; Chen et al. 2015). Finding ways to modulate the behavior or phenotype of macrophages may provide a route to identify novel drug targets for nerve regeneration.

Mokarram et al. (2012) demonstrated that treatment with interferon-gamma (IFN- γ) or interleukin-4 (IL-4) modulated macrophage phenotype toward either M1 or M2a and M2c, respectively. An increase in the M2a and M2c phenotype resulted in faster axonal growth in a rat sciatic nerve gap injury (15 mm). Overall the study showed that the phenotype of the macrophages had a greater effect on regeneration than the extent of their presence (Mokarram et al. 2012).

Interferon-beta (IFN- β) administered to a mouse sciatic nerve crush injury has also demonstrated beneficial effects on axonal growth and motor function recovery (Zanon et al. 2010). IFN- β is a protein produced by fibroblasts which upregulates the expression of major histocompatibility complex class I (MHC class I). In a nerve injury, this increase in MHC class I resulted in an increased expression of S100, GAP-43 and p75^{NTR} coupled with a greater number of regenerated axons (Zanon et al. 2010).

3 Molecular Signaling Pathways

Recent studies have elucidated the effect of signaling pathway activation and the consequent changes in gene expression on nerve regeneration (Fig. 1) (Hiraga et al. 2006; Tedeschi 2011).

A commonly studied excitatory intracellular messenger is cyclic adenosine monophosphate (cAMP) which accumulates following a PNI and acts as an inducer for axon regeneration via several pathways (Raff et al. 1978; Knott et al. 2014; Stewart et al. 1991). cAMP leads to the formation of protein kinase A (PKA), a kinase that blocks the inhibitory effects of myelin-associated glycoprotein (MAG) by blocking the Rho A GTPase in the Rho A inhibitory pathway (Figs. 1 and 2). This results in the assembly of cytoskeleton proteins which are essential for axon elongation. The upregulated PKA and cAMP also have a second effect inducing arginase-1 activity via the phosphorylation of cAMP response element binding protein (CREB). This activity promotes polyamine synthesis which acts to overcome the growth inhibitory effects of MAG (Chan et al. 2014).

The Ras-ERK signaling pathway (Fig. 2) is another commonly studied excitatory pathway. The extracellular signal-regulated kinase (ERK) plays a role in neurite outgrowth and axon survival by activating anti-apoptotic proteins including B-cell lymphoma 2 (Bcl-2) (Chan et al. 2014). The ERK pathway is activated through binding

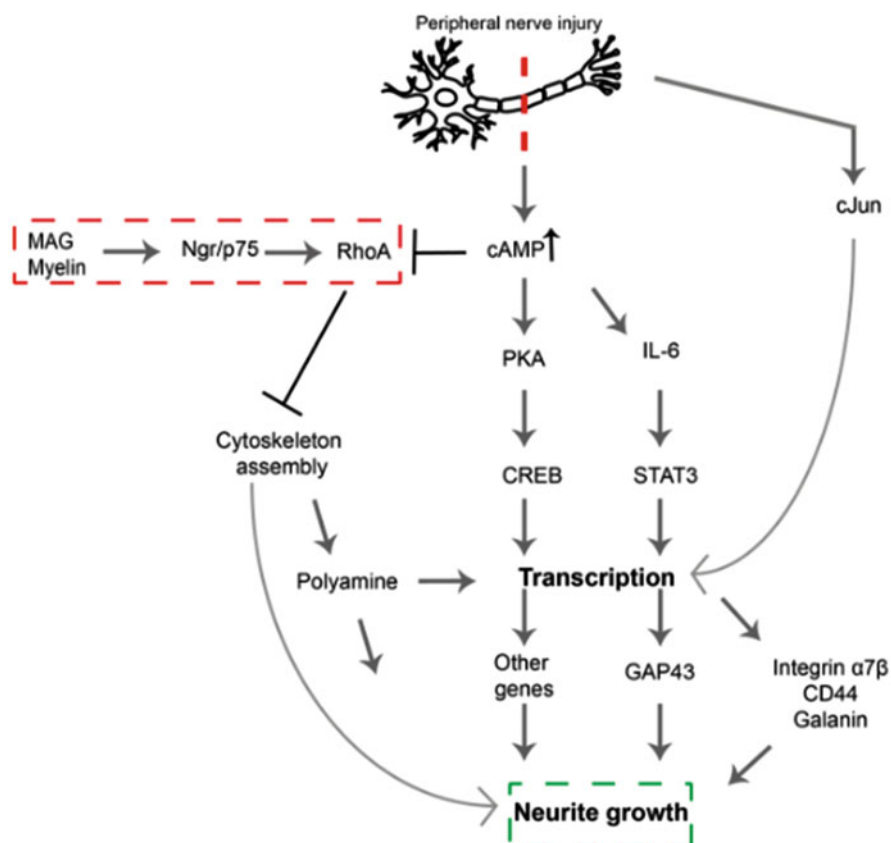


Fig. 1 Signaling pathways activated following peripheral nerve injury. All pathways are excitatory acting to enhance axonal growth except the Rho/ROCK inhibitory pathway indicated in red. Modified from Chen et al. (2007) with permission of Annual Reviews, Inc.; permission conveyed through Copyright Clearance Center, Inc.

of neurotrophins to the tyrosine kinase (Trk) receptor. This leads to Ras activation and subsequent initiation of the tyrosine-threonine kinase MAP/ERK kinase (MEK) and ERK cascade, which encourages neurite growth (Fig. 2) (Klesse et al. 1999).

Phosphatidylinositol-3 kinase (PI3K) is activated by nerve growth factor (NGF) binding to tropomyosin related kinase (Trk) receptors. This results in the phosphorylation of downstream proteins, such as Akt. The Akt signaling pathway activates trophic factors to support the neuron and prevent apoptosis; as a result this cascade is vital for neuron survival (Dudek et al. 1997). Akt participates in other pathways to encourage growth, differentiation, and directional signaling. The inactivation of glycogen synthase kinase 3 β (GSK-3 β) acts to set free the machinery for cytoskeletal assembly and neurite growth (Chan et al. 2014).

responsiveness to the pathway following PNI (Joshi et al. 2015; Wood and Mackinnon 2015).

3.1 Rho/ROCK Signaling Pathway

Activation of the GTPase Rho (or Rho A) to its GTP-bound form with the help of downstream effector kinase ROCK leads to stiffening of the actin cytoskeleton. This initializes changes in the signaling of many downstream effectors, inhibiting axonal elongation and mediating growth cone collapse (Fig. 3) (Chan et al. 2014; Hiraga et al. 2006; Madura et al. 2004).

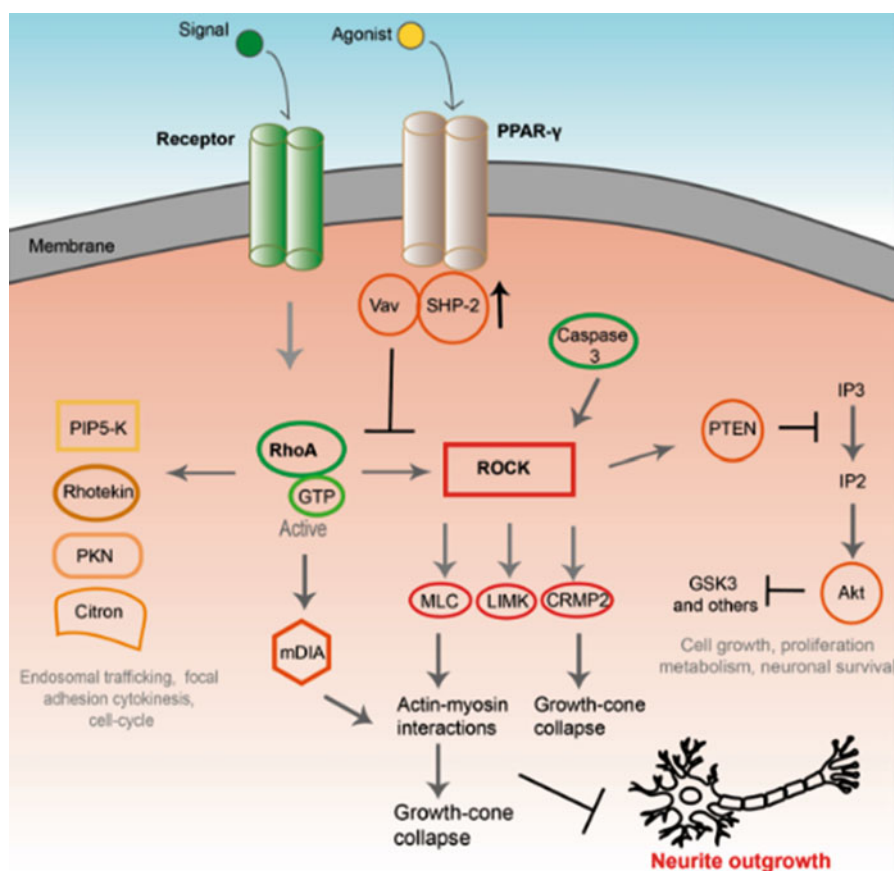


Fig. 3 Overview of the Rho/ROCK inhibitory pathway and its potential targets for drug treatments. (Peroxisome proliferator associated receptor gamma (PPAR-γ), protein tyrosine phosphatase-2 (SHP-2), myosin light chain (MLC), LIM kinase (LIMK), collapsin response mediator protein-2 (CRMP2), protein kinase B (Akt), phosphatase and tensin homolog (PTEN), inositol trisphosphate (IP3)).

The Rho/ROCK pathway (Fig. 3) can also be modulated through activation of upstream receptors, such as the receptor tyrosine kinases; Eph, G-protein coupled receptors, plexins, ApoER2 (Schmandke et al. 2007), and the peroxisome proliferator-activated receptor gamma (PPAR- γ) (Wakino et al. 2004), and through the action of neurogenic inhibitors such as MAG, Nogo-A, and chondroitin sulfate proteoglycans (CSPG). These inhibitory signals appear to converge on the Rho GTPase pathway, ultimately blocking effective regeneration (Tang 2003; Wood and Mackinnon 2015) and providing additional targets for therapy. As the Rho/ROCK signaling pathway appears to act as a nexus for opposing nerve regeneration, it has attracted considerable interest and several potential targets for drug therapies have been identified. The success of such treatments will be dependent on their ability to inhibit the downstream activity of Rho on actin cytoskeleton remodeling in the growth cones, which is essential for axonal regrowth (Joshi et al. 2015; Tang 2003).

In recent years, many agents that target the Rho/ROCK pathway have been identified and tested within PNI models. A number of these agents have demonstrated promising effects, for example, increased axonal outgrowth and improved functional recovery. Furthermore, there has been substantial research conducted using small molecules targeting upstream receptors acting upon the Rho/ROCK pathway, to enhance regeneration following PNI.

The most commonly studied upstream receptor is PPAR- γ , a member of the nuclear receptor family that heterodimerises with retinoic acid-X receptors and is rendered transcriptionally active by binding to a specific DNA sequence element termed the PPAR response element (Wakino et al. 2004; Quintanilla et al. 2014). Activation of PPAR- γ subsequently leads to inhibition of the Rho/ROCK pathway via upregulation of the protein tyrosine phosphatase, Src homology region 2-containing protein tyrosine phosphatase-2 (SHP-2). This cytosolic protein tyrosine phosphatase (PTP) dephosphorylates the Rho-GEF Vav, inactivating it thus suppressing the conversion of inactive GDP-Rho to active GTP-Rho and ultimately resulting in the suppression of Rho/ROCK activity (Wakino et al. 2004).

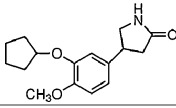
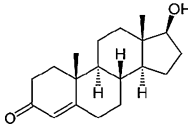
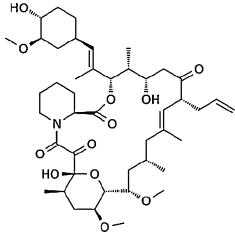
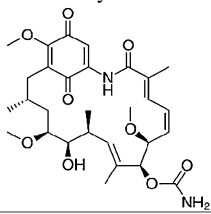
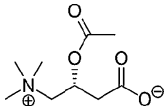
4 Drugs Currently in Research Targeting Commonly Studied Pathways

See Table 1.

Details of many drugs and their effects in PNI studies to date are extensively reviewed by Bota and Fodor (2019), and so only a brief summary of the most commonly studied drugs in the discussed pathways is given here.

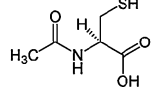
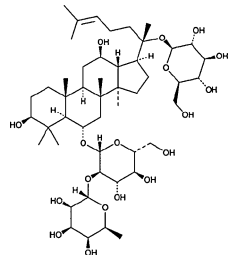
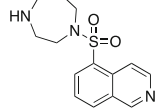
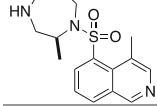
Rolipram is a selective inhibitor of phosphodiesterase (PDE) type 4 receptors which prevents cAMP degradation (Chan et al. 2014; Bota and Fodor 2019). In a nerve transection model in the rat perineal nerve, the administration of rolipram increased the numbers of regenerated motor and sensory neurons 1–2 weeks after injury (Udina et al. 2010). However, rolipram causes significant nausea when administered systemically which limits its translation to the clinic (Chan et al.

Table 1 Studies that have explored the regenerative capacity of drugs targeting signaling pathways following PNI

Compound and chemical structure	Mechanism of action in PNI	Reference
cAMP/PKA pathway		
Rolipram 	Inhibits cAMP phosphodiesterase type 4 receptors	(Pearse et al. 2004) (Udina et al. 2010)
Testosterone 	Trophic effects	(Jones et al. 1997) (Kujawa et al. 1989) (Sharma et al. 2009) (Tetzlaff et al. 2007)
Ras/ERK pathway		
Tacrolimus (FK506) 	Activation of ERK pathway, activation of GAP43 and TGF- β 1	(Fansa et al. 1999) (Azizi et al. 2012) (Gold and Zhong 2004) (Yan et al. 2012) (Rustemeyer et al. 2010) (Sulaiman et al. 2002) (Tajdaran et al. 2019) (Gold et al. 1994) (Labroo et al. 2016) (Labroo et al. 2017)
Geldanamycin 	Activation of ERK pathway, activation of GAP43	(Jin and Sano 2008) (Sun et al. 2012) (Manaenko et al. 2010)
Acetyl-L-carnitine 	Increases ERK1/2 and neurotrophins	(Barhwal et al. 2008) (Hart et al. 2002) (Karsidag et al. 2012) (Kokkalis et al. 2009)

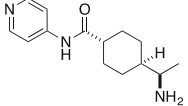
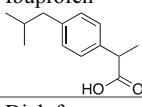
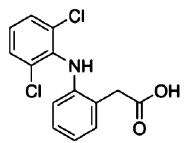
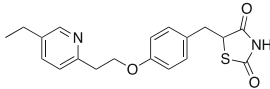
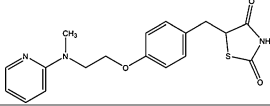
(continued)

Table 1 (continued)

Compound and chemical structure	Mechanism of action in PNI	Reference
		(Kostopoulos et al. 2009) (Wilson et al. 2003) (Wilson et al. 2010) (Wilson et al. 2007)
N-acetyl-cysteine 	Activates Ras-ERK and JAK-STAT pathways.	(Reid et al. 2009) (Yan and Greene 1998) (Hart et al. 2004) (Moschou et al. 2008) (Welin et al. 2009) (Zhang et al. 2005)
Ginsenoside Re 	Increases the expression of GAP43 and S100	(Wang et al. 2015)
PI3K/Akt pathway		
Erythropoietin (Protein)	Increases calcitonin gene-related peptide (CGRP)	(Elfär et al. 2008) (Hoke and Keswani 2005) (Sundem et al. 2016)
Rho/ROCK pathway		
Fasudil (HA-1077) 	Inhibits ROCK and prevents growth cone collapse	(Cheng et al. 2008) (Madura et al. 2007) (Xiao et al. 2014)
Dimethyl-Fasudil (HA-1152) 	Inhibits ROCK and prevents growth cone collapse	(Fuentes et al. 2008) (Lie et al. 2010)

(continued)

Table 1 (continued)

Compound and chemical structure	Mechanism of action in PNI	Reference
Y-27632 	Inhibits ROCK and prevents growth cone collapse	(Joshi et al. 2015) (Fuentes et al. 2008)
Chondroitinase ABC (Enzyme)	Degrades CSPGs and inactivates RhoA	(Udina et al. 2010) (Krekoski et al. 2001) (Zuo et al. 2002)
Ibuprofen 	Strong partial PPAR- γ agonist	(Madura et al. 2011) (Rayner et al. 2018)
Diclofenac 	Partial PPAR- γ agonist	(Mohammadi et al. 2013)
Pioglitazone 	Highly selective PPAR- γ agonist	(Eto et al. 2008)
Rosiglitazone 	Highly selective PPAR- γ agonist	(Chiang et al. 2014)

Inclusion criteria included selected studies that have used drugs targeting the cAMP-PKA, Ras/ERK, PI3K/Akt, and Rho/ROCK pathway for nerve regeneration. Studies exploring outcome measures such as neuropathic pain or regeneration in spinal cord injuries were excluded.

2014). The enzyme chondroitinase ABC (ChABC) degrades chondroitin sulfate proteoglycans that inhibit neurite outgrowth. Udina et al. (2010) administered rolipram and ChABC together following a conduit repair in a common peroneal nerve injury. The combination treatment had no enhanced effect on nerve regeneration, demonstrating that only one of the signaling pathways needs to be blocked to improve axon growth (Bota and Fodor 2019; Udina et al. 2010).

Tacrolimus (FK506) is an immunosuppressant that binds to FK binding proteins to inhibit T-cell function and reduce inflammatory cytokines by inhibiting the calcineurin and calmodulin-dependent phosphatase (Mekaj et al. 2014). The mechanism through which neuroregenerative effects are thought to manifest is via its interaction with the binding to FK protein-52 (Gold et al. 1999). These two actions lead to the reduction in scar formation and activation of the ERK pathway (Bota and

Fodor 2019). Many *in vitro* and *in vivo* studies have demonstrated the regenerative properties of tacrolimus. Tacrolimus has been shown to significantly increase neurite outgrowth in SH-SY5Y neuroblastoma cells (Gold et al. 1999) and dorsal root ganglia cells from rat and chick embryos (Labroo et al. 2016; Labroo et al. 2017; Tajdaran et al. 2015). *In vivo*, tacrolimus was found to increase the rate of regeneration and improved functional recovery in transection injuries (Yan et al. 2012; Gold et al. 1994, 1995; Jost et al. 2000), crush injuries (Jost et al. 2000; Lee et al. 2000), and allograft nerve repairs (Rustemeyer et al. 2010). As with Rolipram, risks associated with systemic administration of tacrolimus present a barrier to clinical translation (Chan et al. 2014). This problem could be addressed by the local delivery of drugs, an area of growing interest in nerve injuries. A recent study demonstrated the use of conduits made from a biocompatible material, polytetrafluoroethylene (PTFE), to deliver tacrolimus locally for PNI to reduce the systemic side effects (Labroo et al. 2017).

Geldanamycin was also tested as an alternative to tacrolimus as it has the same common binding protein but is non-immunosuppressive. In a rat nerve crush injury Geldanamycin improved axonal regeneration and functional recovery, but not to the same extent as tacrolimus (Sun et al. 2012).

Erythropoietin (EPO) is a renal cytokine that is produced by cells in the nervous system and activates the EPO-receptor which is found in glial cells. Administration following a sciatic nerve crush injury in mice resulted in a myeloprotective effect leading to faster functional recovery (Sundem et al. 2016). EPO also enhanced functional and histological recovery 8 weeks after surgery following a sciatic nerve injury repaired with a silicone conduit (Yin et al. 2010).

Fasudil is a ROCK inhibitor which is approved for use in the clinic; it is marketed in Japan as a treatment for cerebral vasospasm after subarachnoid hemorrhage and it was also evaluated for use in patients with stable angina pectoris (Nishio et al. 2006). It has also demonstrated beneficial effects in other clinical applications such as CNS injury, oncology, bronchial asthma, pulmonary hypertension, glaucoma, preterm labor, erectile dysfunction, and renal disease (Mueller et al. 2005; Olson 2008). Fasudil is a moderate inhibitor of ROCK ($K_i = 330$ nM) (Mueller et al. 2005) and has shown potential in promoting axonal growth (Cheng et al. 2008; Lingor et al. 2007) and improving functional outcomes in models of peripheral nerve injury (Madura et al. 2007).

Dimethyl-fasudil (HA-1152), a derivative of Fasudil, has an increased affinity for ROCK ($K_i = 1.6$ nM) (Mueller et al. 2005), but despite this has only been studied *in vitro* using neuronal cell cultures. These studies demonstrated increased neurite outgrowth relative to the parent compound, suggesting that dimethyl-fasudil has the potential to be taken forward in animal studies to determine functional recovery as well as regeneration following PNI (Fuentes et al. 2008; Lie et al. 2010).

Small molecules that target the PPAR- γ receptor have also been investigated for their potential to promote peripheral nerve regeneration. Agonists for this receptor usually contain a lipophilic backbone and an acidic moiety, usually a carboxylate (Puhl et al. 2015), a pharmacophore found in many nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs are well-characterized modulators of PPAR- γ and have demonstrated different degrees of partial agonism (Puhl et al. 2015).

To date promising results have been reported for treatment with ibuprofen and diclofenac following PNI. For example, Madura et al. (2011) demonstrated an improvement in functional recovery and remyelination of axons after 3 months following 3 weeks ibuprofen treatment in a rat tibial nerve transection model. Furthermore, delivery of diclofenac to a transected rat sciatic nerve via an artery graft elicited improvement in both functional recovery and axon regeneration (Mohammadi et al. 2013).

To the best of our knowledge, other NSAIDs have yet to be investigated in PNI models, despite demonstrated affinity for PPAR- γ (Puhl et al. 2015). Given the promising results obtained with ibuprofen and diclofenac, further investigation of NSAIDs as agents to promote regeneration may prove fruitful. It is likely that greater understanding of how NSAIDs interact with PPAR- γ will help the development of more effective drugs targeting this receptor for the treatment of PNI (Lezana et al. 2016).

Another class of drugs that act upon PPAR- γ is the glitazones which are used clinically as antidiabetics. Pioglitazone and rosiglitazone have been studied for use in PNI. Pioglitazone demonstrated improved myelination following 3 weeks treatment in a crush injury model in the sciatic nerve in mice (Eto et al. 2008), further supporting the proposal of PPAR- γ as a promising target for PNI.

Many other drugs have been studied for use in the treatment of PNI including hormones, neurological drugs, growth factors, vitamins, and traditional Chinese medicines (Bota and Fodor 2019).

5 Drug Screening

Preclinical model systems including in vitro cell cultures and animal studies are fundamental in the discovery and development of new pharmaceutical therapies (Breyer et al. 2015). Primary compound screening in humans raises obvious ethical and regulatory concerns and so effective and appropriate model systems are paramount to establish efficacy and safety before they are taken forward into clinical trials (Breyer et al. 2015). However, studies have demonstrated that there are difficulties in correlating the effects seen in animal and human studies. This means that animal models (see chapter ► [“Appropriate Animal Models for Translational Nerve Research”](#)) are not always accurate predictors of therapeutic effects in humans, which in turn contributes to the high failure rate of clinical trials (Seok et al. 2013; Suckling 2008). Therefore, there is a clear need for the development and validation of cellular and animal models that can harmonize with the behavior seen in human PNI. This is becoming easier as our understanding of the molecular basis of pathogenesis improves (Breyer et al. 2015).

To date there is limited literature on cell culture models specifically designed to screen new potential drugs for PNI (Rayner et al. 2018). In PNI the synergistic relationship between neurons and Schwann cells plays a key role in nerve regeneration; therefore, it is desirable to recreate this key cell-to-cell interaction in a

model when screening the effects of drug therapies. Monolayer culture and animal models are widely used but to overcome their various limitations more 3-dimensional (3D) *in vitro* models are being developed as an alternative, for example, explant cultures, self-assembling aggregate cultures, and scaffold-based cell cultures (Ko and Frampton 2016). Various 3D culture model systems have been adopted by the biomaterials and tissue engineering communities to study the influence of extracellular matrices in neurite formation and growth, but these tend not to have been developed specifically for compound screening to identify new drugs (Ahmed et al. 2006; Baldwin et al. 1996; Bozkurt et al. 2007; Herbert et al. 1998; Kofron et al. 2009; Kraus et al. 2015; Pittier et al. 2005). A 3D co-culture engineered tissue model to screen drugs specifically for PNI has been developed, to allow the exploration of key regenerative events such as neurite formation and optimal dosing regimens and the mechanism of action of drug candidates (Rayner et al. 2018). The development of effective 3D *in vitro* models potentially enables researchers to bridge the gap between monolayer *in vitro* models and *in vivo* studies more successfully (Rayner et al. 2018; Hopkins et al. 2015), potentially advancing the preclinical development process and the subsequent success of clinical trials (Ko and Frampton 2016).

Regardless of advancements made in the development of reliable *in vitro* models to screen therapies for PNI, animal models still play a key part in preclinical studies as they remain a mandatory requirement of regulatory bodies (Geuna et al. 2016). To ensure the successful translation of drug compounds from the bench to the clinic, animal models need to enable a reliable prediction of human pathophysiology (Greek 2012).

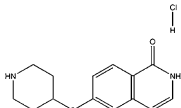
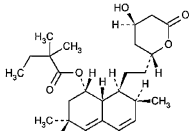
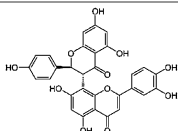
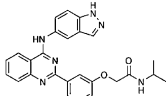
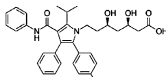
For the study of PNI, animal models allow the additional evaluation of functional recovery and provide a holistic view of peripheral nerve recovery and whole-body interactions (Donaldson and Hoke 2014). Animal studies can permit the study of cellular and molecular changes, which is highly important in the field of PNI, as successful recovery relies on an orchestrated permissible cell environment (Allodi et al. 2012). This includes the influence of the Schwann cells and the impact of a local immune and inflammatory response (Gingras et al. 2003). Considering all of these factors, it is therefore vital to choose a model that answers a specific question and uses appropriate methods to derive meaningful conclusions (Navarro 2016; Denayer et al. 2014).

Rodents are commonly used in PNI research as they are easily housed and handled, inexpensive, and large numbers of genetically identical animals can be obtained. Many different nerves in the rat are used for PNI studies; however, the most commonly used is the sciatic nerve (Varejao et al. 2004). Its size ensures easy surgical access and helps facilitate microsurgical injury and repair and is equivalent to the human digital nerve making it a useful model in testing human-scale repair approaches. In addition, the rat sciatic nerve is a useful model to assess motor function recovery as there are several established automated behavioral tests available (Tos et al. 2009). Finally, using the rat sciatic nerve model means the data are comparable to most of the previous literature in the PNI field (Tos et al. 2009; Angius et al. 2012).

6 Repurposing Drugs for PNI

Drug discovery and development is expensive and time consuming with a high risk of failure; therefore, more companies are adopting drug repurposing as an alternative (Padhy and Gupta 2011). This technique employs data mining, bioinformatics, and other screening platforms to identify drugs that are already approved for other clinical indications and to reposition them for new applications (Padhy and Gupta 2011). This process allows the innovative use of existing drugs for neglected diseases (Padhy and Gupta 2011). Many drugs identified for research in nerve regeneration have been the result of this repurposing approach. For example, agents targeting the Rho/Rock pathway used for other clinical conditions have already shown benefit in nerve regeneration in preclinical studies. Many of these studies have involved treating CNS injuries and so through the same mechanism of action Rho/ROCK inhibitors can be applied to the PNS. Furthermore, available drugs and research compounds used for other common conditions have been shown to mediate the Rho/ROCK pathway. Some examples are summarized in Table 2.

Table 2 Compounds that target the Rho/ROCK pathway for other clinical indications.

Drug name	Chemical structure	Clinical indication	Predicted target in PNS	Citation
AMA0076	(Undisclosed)	Glaucoma	ROCK	(Van De Velde et al. 2014)
SAR407899		Renal vascular resistance	ROCK	(GRISK et al. 2012)
Statins (Class)		Hypercholesterolaemia	PPAR-alpha	(Martin et al. 2001)
Morelloflavona		Cancer	Rho GTPases	(Pang et al. 2009)
SLx-2119		Anti-fibrotic	ROCK	(Boerma et al. 2008)
Atorvastatin		Anti-fibrotic	ROCK	(Boerma et al. 2008)

Studies that have explored the pharmacological activity of drugs that target the Rho/ROCK pathway directly in other clinical indications, excluding nerve injuries. Inclusion criteria included studies that have studied drugs targeting Rho, ROCK PPAR-gamma SHP-2 or VAV.

7 Drug Delivery

Small molecules that have been identified for the treatment of PNI are associated with potentially damaging side effects when administered systemically, which has restricted clinical translation (Yang and Pierstorff 2012). These side effects can be reduced through decreasing drug concentrations; however, this may reduce the therapeutic efficacy of the drug and is not a sustainable solution (Yang and Pierstorff 2012). Therefore, the development of local drug delivery platforms could be advantageous not just in reducing side effects but also for drugs with physicochemical issues such as nonoptimal solubility.

Research into the use of local drug delivery platforms in PNI research is lacking. No study has been published in which a suitable treatment duration for any agent has been identified; however, as regeneration of nerves in humans takes a remarkable time (months-years), treatment durations may need to extend to this amount of time. Long term oral treatment can lead to side effects but also problems with patient compliance. Therefore, a device that can deliver drugs locally to the injured nerve over a sustained period could be beneficial.

Many advancements have been made in targeted therapeutics for PNI to deliver cells, proteins, platelet-rich plasma, and gene therapy to promote nerve regeneration (Federici et al. 2007; Kuffler 2014; Bhangra et al. 2016; Busuttill et al. 2017). These therapies are delivered through the use of conduits that are surgically inserted into the injury site. These interventions have proven beneficial in promoting nerve regeneration via the induction of axon outgrowth and neurological recovery (Kuffler 2014). As other therapeutics have been successfully delivered locally through conduits, this provides a platform that could be feasible for drug therapies.

Tissue engineering approaches have developed synthetic and natural biomaterials as alternative strategies to nerve grafting for the surgical treatment of PNI (Subramanian et al. 2009). These materials could not only provide a new approach for delivering small molecules but also the possibility of dual therapies, delivering drug treatments alongside cell or gene therapies within the same device. The materials are biocompatible and include synthetic polymers, or materials that occur naturally in the body such as collagen and fibrin. These materials have the potential to provide a controlled-release delivery device that would naturally biodegrade in the body over time. As a result this makes the therapy less invasive for the patient, by eradicating the need for additional surgery to remove the device after treatment. Biodegradable polymers possess weak bonds such as ester, amide, and anhydride bonds that break down by hydrolysis or enzyme degradation (Fu and Kao 2010). On the other hand, nondegradable polymers are still extensively used for drug delivery (Liechty et al. 2010) for other clinical applications due to their robust structure, durability, mechanical strength, and relatively slow release, so these may also be a feasible option for PNI.

Extensive work has been conducted to develop both degradable and non-degradable biomaterials to deliver drugs to other tissues for various clinical conditions. Most of these controlled-release systems have demonstrated biocompatibility and effectiveness which can be tested for application in a neural environment. They

need to possess certain properties to be used clinically such as chemically inert, free of leachable impurities, have a suitable physical structure, be readily processable, and the incorporated drug needs to have long term stability (Brannon-Peppas 1997; Liechty et al. 2010; Pillai and Panchagnula 2001).

8 Clinical Trials

To conduct a successful clinical trial for a drug therapy for PNI, there needs to be efficient measures to analyze and quantify regeneration. This is difficult as the clinical experience in humans involves many outcomes which are subjective and not easily quantifiable. Assessing recovery remains challenging for therapists and surgeons as they require measures that can aid clinical diagnosis, assess and compare surgical repair techniques, track rehabilitation progress, provide feedback to both patient and therapist, as well as ascertain disability after injury. The outcomes of recovery are usually disproportionate to the preinjury function and the individual patient's expectations differ (Quick et al. 2016).

The two most commonly used assessment tools for PNI are the Visual Analogue Score (VAS) for pain assessment and the Medical Research Council (MRC) motor assessments which are far from ideal. Motor function such as muscle contraction is the simplest method to conduct which provides objective and quantifiable outcomes. Subjective assessments of individual muscle functions are rare and usually involve measuring the peak force a muscle can generate; however, these assessments are influenced by other aspects of the recovery such as the underlying physiology and other injuries or other medical conditions which can confound the results. Furthermore, patients usually report that the scores recorded from these tests do not translate to their day to day functional abilities and so the results do not necessarily represent the patient's experience. In addition, it has been found that the patient's engagement, pain threshold, and kinesophobia can strongly influence the outcome and so inter-variability between patients will be high.

Another objective assessment is movement which can be easily observed and graded. However, it could be argued that the observation of a movement is not more reliable than the subjective experience of a movement. Motion capture (MoCap) technologies are a feasible option to analyze gait in the clinic as they can measure movement patterns and interpret these patterns (Baker 2006; Marin et al. 2019). In biomedical research, the gold standard is optical technology which tracks reflective surface markers with infrared cameras (Marin et al. 2019). MoCap is widely used in clinical research as it can generate individual objective outcome measures; however, there are associated challenges with simplicity and usability that have prevented translation for clinical rehabilitation (Benedetti et al. 2017; Mulder et al. 1998). As the systems are highly complex, they need a technically accurate protocol that is reproducible and a system that can process a huge amount of data. This leads to the requirement for the professional using the equipment to be highly trained. More research is required for a more simplistic, effective method for clinical application (Marin et al. 2019).

It is accepted that the outcomes for human peripheral nerve regeneration are inferior to those used in rodent models; however, it is relatively easy to analyze regeneration and muscle re-innervation in animal models. Tissue can be obtained to quantify muscle mass and the number of axons can be determined histologically as a means to enumerate regeneration. For obvious reasons, this is not plausible in humans and therefore other methods are required (Rayner et al. 2019).

There is a lack of drugs entering clinical trials for PNI; however, a pilot Phase 2 study was conducted to determine the effects of tacrolimus on the repair of ulnar, median, or sciatic nerve injury in patients, with tacrolimus treatment advancing the outcome of Tinel's sign (Phan and Schuind 2012). Another phase 2 study is currently under recruitment to test the efficacy of tesamorelin, a synthetic form of growth hormone releasing hormone, in motor and sensory function following PNI (ClinicalTrials.gov 2019b). Furthermore, a phase 4 study will be recruiting to determine the role of voltage-gated potassium channel blocker 4-aminopyridine (4-AP) to treat a peripheral nerve traction or crush injury (ClinicalTrials.gov 2019a).

Tacrolimus has been suggested to exert its regenerative effect through the activation of the ERK pathway (Gold and Zhong 2004); however, the other drugs that have reached clinical trials for use in PNI act through different mechanisms than the aforementioned approaches in this chapter. Tesamorelin is a synthetic form of growth-hormone-releasing hormone (GHRH) which stimulates the synthesis of growth hormone and increases insulin-growth factor and 4-aminopyridine (dalfampridine) is a potassium channel blocker which has shown to increase conduction of action potentials in demyelinated axons (Dunn and Blight 2011; Tseng et al. 2016). This indicates that there are many other clinical approaches available as additional options in improving recovery.

9 Conclusions

Although there have been many advancements made in the identification of targets for pharmacological agents to treat nerve injury, the field is still in its infancy. To date only a few agents have been directly tested in humans for applications related to peripheral nerve function. Ibuprofen as well as other NSAIDs are routinely used for inflammation and pain and already have well-established safety and efficacy profiles. Therefore, it would be feasible to test such drugs in humans following nerve injury with a low risk of toxic effects.

Furthermore, the need for extensive preclinical studies can be a drawback in the development of new drugs for treating nerve injuries, making the repurposing of established drugs an attractive alternative proposition. Advances in preclinical testing of potential drug therapies, combined with development of clinical trial protocols and relevant outcome measures, provide an exciting opportunity to translate new pharmacological nerve injury treatments into clinical application.

Future work would also need to address the issues associated with method and site of drug administration. Recent studies have suggested the use of conduits to

deliver drugs locally to the site of injury, which may overcome this problem, offering an exciting alternative to systemic administration.

References

- Ahmed I, Liu HY, Mamiya PC, Ponery AS, Babu AN, Weik T, Schindler M, Meiners S (2006) Three-dimensional nanofibrillar surfaces covalently modified with tenascin-C-derived peptides enhance neuronal growth in vitro. *J Biomed Mater Res A* 76:851–860
- Allodi I, Udina E, Navarro X (2012) Specificity of peripheral nerve regeneration: interactions at the axon level. *Prog Neurobiol* 98:16–37
- Angius D, Wang H, Spinner RJ, Gutierrez-Cotto Y, Yaszemski MJ, Windebank AJ (2012) A systematic review of animal models used to study nerve regeneration in tissue-engineered scaffolds. *Biomaterials* 33:8034–8039
- Azizi S, Mohammadi R, Amini K, Fallah R (2012) Effects of topically administered FK506 on sciatic nerve regeneration and reinnervation after vein graft repair of short nerve gaps. *Neurosurg Focus* 32:E5
- Baker R (2006) Gait analysis methods in rehabilitation. *J Neuroeng Rehabil* 3:4
- Baldwin SP, Krewson CE, Saltzman WM (1996) PC12 cell aggregation and neurite growth in gels of collagen, laminin and fibronectin. *Int J Dev Neurosci* 14:351–364
- Barhwal K, Hota SK, Prasad D, Singh SB, Ilavazhagan G (2008) Hypoxia-induced deactivation of NGF-mediated ERK1/2 signaling in hippocampal cells: neuroprotection by acetyl-L-carnitine. *J Neurosci Res* 86:2705–2721
- Benedetti MG, Beghi E, De Tanti A, Cappozzo A, Basaglia N, Cutti AG, Cereatti A, Stagni R, Verdini F, Manca M, Fantozzi S, Mazza C, Camomilla V, Campanini I, Castagna A, Cavazzuti L, Del Maestro M, Croce UD, Gasperi M, Leo T, Marchi P, Petrarca M, Piccinini L, Rabuffetti M, Ravaschio A, Sawacha Z, Spolaor F, Tesio L, Vannozzi G, Visintin I, Ferrarin M (2017) SIAMOC position paper on gait analysis in clinical practice: general requirements, methods and appropriateness. Results of an Italian consensus conference. *Gait Posture* 58:252–260
- Benito C, Davis CM, Gomez-Sanchez JA, Turmaine M, Meijer D, Poli V, Mirsky R, Jessen KR (2017) STAT3 controls the long-term survival and phenotype of repair Schwann cells during nerve regeneration. *J Neurosci* 37:4255–4269
- Bhangra KS, Busuttil F, Phillips JB, Rahim AA (2016) Using stem cells to grow artificial tissue for peripheral nerve repair. *Stem Cells Int* 2016:7502178
- Boerma M, Fu Q, Wang J, Loose DS, Bartolozzi A, Ellis JL, McGonigle S, Paradise E, Sweetnam P, Fink LM, Vozenin-Brotans MC, Hauer-Jensen M (2008) Comparative gene expression profiling in three primary human cell lines after treatment with a novel inhibitor of Rho kinase or atorvastatin. *Blood Coagul Fibrinolysis* 19:709–718
- Bota O, Fodor L (2019) The influence of drugs on peripheral nerve regeneration. *Drug Metab Rev* 51:1–27
- Bozkurt A, Brook GA, Moellers S, Lassner F, Sellhaus B, Weis J, Woeltje M, Tank J, Beckmann C, Fuchs P, Damink LO, Schugner F, Heschel I, Pallua N (2007) In vitro assessment of axonal growth using dorsal root ganglia explants in a novel three-dimensional collagen matrix. *Tissue Eng* 13:2971–2979
- Brannon-Peppas L (1997) Polymers in controlled drug delivery. MD+DI Qmed. Medical Plastics and Biomaterials Magazine, Santa Monica, California
- Breyer MD, Look AT, Cifra A (2015) From bench to patient: model systems in drug discovery. *Dis Model Mech* 8:1171–1174
- Burnett MG, Zager EL (2004) Pathophysiology of peripheral nerve injury: a brief review. *Neurosurg Focus* 16:E1
- Busuttil F, Rahim AA, Phillips JB (2017) Combining gene and stem cell therapy for peripheral nerve tissue engineering. *Stem Cells Dev* 26:231–238

- Chan KM, Gordon T, Zochodne DW, Power HA (2014) Improving peripheral nerve regeneration: from molecular mechanisms to potential therapeutic targets. *Exp Neurol* 261:826–835
- Chen ZL, Yu WM, Strickland S (2007) Peripheral regeneration. *Annu Rev Neurosci* 30:209–233
- Chen P, Piao X, Bonaldo P (2015) Role of macrophages in Wallerian degeneration and axonal regeneration after peripheral nerve injury. *Acta Neuropathol* 130:605–618
- Cheng C, Webber CA, Wang J, Xu Y, Martinez JA, Liu WQ, McDonald D, Guo GF, Nguyen MD, Zochodne DW (2008) Activated RHOA and peripheral axon regeneration. *Exp Neurol* 212:358–369
- Chiang MC, Cheng YC, Chen HM, Liang YJ, Yen CH (2014) Rosiglitazone promotes neurite outgrowth and mitochondrial function in N2A cells via PPARgamma pathway. *Mitochondrion* 14:7–17
- Clinicaltrials.gov (2019a) 4-aminopyridine treatment for nerve injury [Online]. U.S. National Library of Medicine. <https://ClinicalTrials.gov/show/NCT03701581>. Accessed 2019
- Clinicaltrials.gov (2019b) Tesamorelin to improve functional outcomes after peripheral nerve injury [Online]. U.S. National Library of Medicine. <https://clinicaltrials.gov/ct2/show/NCT03150511?cond=nerve+injury&draw=3&rank=4>. Accessed 2019
- Demidenko ZN, Blagosklonny MV (2009) At concentrations that inhibit mTOR, resveratrol suppresses cellular senescence. *Cell Cycle* 8:1901–1904
- Denayer T, Stohr T, Roy MV (2014) Animal models in translational medicine: validation and prediction. *New Horiz Transl Med* 2:5–11
- Deumens R, Bozkurt A, Meek MF, Marcus MA, Joosten EA, Weis J, Brook GA (2010) Repairing injured peripheral nerves: bridging the gap. *Prog Neurobiol* 92:245–276
- Donaldson K, Hoke A (2014) Studying axonal degeneration and regeneration using in vitro and in vivo model: the translational potential. *Future Neurol* 9:461–473
- Dubovy P, Jancalek R, Kubek T (2013) Role of inflammation and cytokines in peripheral nerve regeneration. *Int Rev Neurobiol* 108:173–206
- Dudek H, Datta SR, Franke TF, Birnbaum MJ, Yao R, Cooper GM, Segal RA, Kaplan DR, Greenberg ME (1997) Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science* 275:661–665
- Dunn J, Blight A (2011) Dalfampridine: a brief review of its mechanism of action and efficacy as a treatment to improve walking in patients with multiple sclerosis. *Curr Med Res Opin* 27:1415–1423
- Elbaz AA, Abu-Almaaty AH, Hassan MK, Mohammed EA, Aziz MM (2017) Therapeutic role of Schwann cells and resveratrol or melatonin combination in peripheral nerve injured rat models. *J Exp Appl Animal Sci* 2:190–210
- Elfar JC, Jacobson JA, Puzas JE, Rosier RN, Zuscik MJ (2008) Erythropoietin accelerates functional recovery after peripheral nerve injury. *J Bone Joint Surg Am* 90:1644–1653
- Eto M, Sumi H, Fujimura H, Yoshikawa H, Sakoda S (2008) Pioglitazone promotes peripheral nerve remyelination after crush injury through CD36 upregulation. *J Peripher Nerv Syst* 13:242–248
- Fansa H, Keilhoff G, Altmann S, Plogmeier K, Wolf G, Schneider W (1999) The effect of the immunosuppressant FK 506 on peripheral nerve regeneration following nerve grafting. *J Hand Surg (Br)* 24:38–42
- Faroni A, Mobasser SA, Kingham PJ, Reid AJ (2015) Peripheral nerve regeneration: experimental strategies and future perspectives. *Adv Drug Deliv Rev* 82–83:160–167
- Federici T, Liu JK, Teng Q, Yang J, Boulis NM (2007) A means for targeting therapeutics to peripheral nervous system neurons with axonal damage. *Neurosurgery* 60:911–918; discussion 911–8
- Fex Sønningsen A, Dahlin LB (2013) Repair of the peripheral nerve-remyelination that works. *Brain Sci* 3:1182–1197
- Fu SY, Gordon T (1995) Contributing factors to poor functional recovery after delayed nerve repair: prolonged axotomy. *J Neurosci* 15:3876–3885
- Fu Y, Kao WJ (2010) Drug release kinetics and transport mechanisms of non-degradable and degradable polymeric delivery systems. *Expert Opin Drug Deliv* 7:429–444

- Fuentes EO, Leemhuis J, Stark GB, Lang EM (2008) Rho kinase inhibitors Y27632 and H1152 augment neurite extension in the presence of cultured Schwann cells. *J Brachial Plex Peripher Nerve Inj* 3:19
- Gaudet AD, Popovich PG, Ramer MS (2011) Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. *J Neuroinflammation* 8:110
- Geuna S, Raimondo S, Fregnan F, Haastert-Talini K, Grothe C (2016) In vitro models for peripheral nerve regeneration. *Eur J Neurosci* 43:287–296
- Gingras M, Bergeron J, Dery J, Durham HD, Berthod F (2003) In vitro development of a tissue-engineered model of peripheral nerve regeneration to study neurite growth. *FASEB J* 17:2124–2126
- Gold BG, Zhong YP (2004) FK506 requires stimulation of the extracellular signal-regulated kinase 1/2 and the steroid receptor chaperone protein p23 for neurite elongation. *Neurosignals* 13:122–129
- Gold BG, Storm-Dickerson T, Austin DR (1994) The immunosuppressant FK506 increases functional recovery and nerve regeneration following peripheral nerve injury. *Restor Neurol Neurosci* 6:287–296
- Gold BG, Katoh K, Storm-Dickerson T (1995) The immunosuppressant FK506 increases the rate of axonal regeneration in rat sciatic nerve. *J Neurosci* 15:7509–7516
- Gold BG, Densmore V, Shou W, Matzuk MM, Gordon HS (1999) Immunophilin FK506-binding protein 52 (not FK506-binding protein 12) mediates the neurotrophic action of FK506. *J Pharmacol Exp Ther* 289:1202–1210
- Gomez-Sanchez JA, Carty L, Iruarizaga-Lejarreta M, Palomo-Irigoyen M, Varela-Rey M, Griffith M, Hantke J, Macias-Camara N, Azkargorta M, Aurrekoetxea I, De Juan VG, Jefferies HB, Aspichueta P, Elortza F, Aransay AM, Martinez-Chantar ML, Baas F, Mato JM, Mirsky R, Woodhoo A, Jessen KR (2015) Schwann cell autophagy, myelinophagy, initiates myelin clearance from injured nerves. *J Cell Biol* 210:153–168
- Greek R (2012) Animal models in drug development. IntechOpen, London
- Grisk O, Schluter T, Reimer N, Zimmermann U, Katsari E, Plettenburg O, Lohn M, Wollert HG, Rettig R (2012) The Rho kinase inhibitor SAR407899 potently inhibits endothelin-1-induced constriction of renal resistance arteries. *J Hypertens* 30:980–989
- Hall SM (1986) Regeneration in cellular and acellular autografts in the peripheral nervous system. *Neuropathol Appl Neurobiol* 12:27–46
- Hall S (2005) The response to injury in the peripheral nervous system. *J Bone Joint Surg Br* 87:1309–1319
- Hart AM, Wiberg M, Youle M, Terenghi G (2002) Systemic acetyl-L-carnitine eliminates sensory neuronal loss after peripheral axotomy: a new clinical approach in the management of peripheral nerve trauma. *Exp Brain Res* 145:182–189
- Hart AM, Terenghi G, Kellerth JO, Wiberg M (2004) Sensory neuroprotection, mitochondrial preservation, and therapeutic potential of N-acetyl-cysteine after nerve injury. *Neuroscience* 125:91–101
- Hart AM, Terenghi G, Wiberg M (2008) Neuronal death after peripheral nerve injury and experimental strategies for neuroprotection. *Neurol Res* 30:999–1011
- Herbert CB, Nagaswami C, Bittner GD, Hubbell JA, Weisel JW (1998) Effects of fibrin micromorphology on neurite growth from dorsal root ganglia cultured in three-dimensional fibrin gels. *J Biomed Mater Res* 40:551–559
- Hiraga A, Kuwabara S, Doya H, Kanai K, Fujitani M, Taniguchi J, Arai K, Mori M, Hattori T, Yamashita T (2006) Rho-kinase inhibition enhances axonal regeneration after peripheral nerve injury. *J Peripher Nerv Syst* 11:217–224
- Hoke A, Brushart T (2010) Introduction to special issue: challenges and opportunities for regeneration in the peripheral nervous system. *Exp Neurol* 223:1–4
- Hoke A, Keswani SC (2005) Neuroprotection in the PNS: erythropoietin and immunophilin ligands. *Ann N Y Acad Sci* 1053:491–501
- Hopkins AM, Desimone E, Chwalek K, Kaplan DL (2015) 3D in vitro modeling of the central nervous system. *Prog Neurobiol* 125:1–25

- Isaacs J (2013) Major peripheral nerve injuries. *Hand Clin* 29:371–382
- Jessen KR, Mirsky R (2016) The repair Schwann cell and its function in regenerating nerves. *J Physiol* 594:3521–3531
- Jessen KR, Mirsky R, Salzer J (2008) Introduction. Schwann cell biology. *Glia* 56:1479–1480
- Jin E, Sano M (2008) Neurite outgrowth of NG108-15 cells induced by heat shock protein 90 inhibitors. *Cell Biochem Funct* 26:825–832
- Jones KJ, Kinderman NB, Oblinger MM (1997) Alterations in glial fibrillary acidic protein (GFAP) mRNA levels in the hamster facial motor nucleus: effects of axotomy and testosterone. *Neurochem Res* 22:1359–1366
- Jones S, Eisenberg HM, Jia X (2016) Advances and future applications of augmented peripheral nerve regeneration. *Int J Mol Sci* 17
- Joshi AR, Bobylev I, Zhang G, Sheikh KA, Lehmann HC (2015) Inhibition of Rho-kinase differentially affects axon regeneration of peripheral motor and sensory nerves. *Exp Neurol* 263:28–38
- Jost SC, Doolabh VB, Mackinnon SE, Lee M, Hunter D (2000) Acceleration of peripheral nerve regeneration following FK506 administration. *Restor Neurol Neurosci* 17:39–44
- Karsidag S, Akcal A, Sahin S, Karsidag S, Kabukcuoglu F, Ugurlu K (2012) Neurophysiological and morphological responses to treatment with acetyl-L-carnitine in a sciatic nerve injury model: preliminary data. *J Hand Surg Eur Vol* 37:529–536
- Klesse LJ, Meyers KA, Marshall CJ, Parada LF (1999) Nerve growth factor induces survival and differentiation through two distinct signaling cascades in PC12 cells. *Oncogene* 18:2055–2068
- Knott EP, Assi M, Pearse DD (2014) Cyclic AMP signaling: a molecular determinant of peripheral nerve regeneration. *Biomed Res Int* 2014:651625
- Ko KR, Frampton JP (2016) Developments in 3D neural cell culture models: the future of neurotherapeutics testing? *Expert Rev Neurother* 16:739–741
- Kofron CM, Fong VJ, Hoffman-Kim D (2009) Neurite outgrowth at the interface of 2D and 3D growth environments. *J Neural Eng* 6:016002
- Kokkalis ZT, Soucacos PN, Terzis JK (2009) Effect of acetyl-L-carnitine on axonal sprouting following donor nerve injury distal to an end-to-side neurorrhaphy model. *J Reconstr Microsurg* 25:483–495
- Kostopoulos VK, Davis CL, Terzis JK (2009) Effects of acetylo-L-carnitine in end-to-side neurorrhaphy: a pilot study. *Microsurgery* 29:456–463
- Kraus D, Boyle V, Leibig N, Stark GB, Penna V (2015) The neuro-spheroid – a novel 3D in vitro model for peripheral nerve regeneration. *J Neurosci Methods* 246:97–105
- Krekoski CA, Neubauer D, Zuo J, Muir D (2001) Axonal regeneration into acellular nerve grafts is enhanced by degradation of chondroitin sulfate proteoglycan. *J Neurosci* 21:6206–6213
- Kuffler DP (2014) An assessment of current techniques for inducing axon regeneration and neurological recovery following peripheral nerve trauma. *Prog Neurobiol* 116:1–12
- Kujawa KA, Kinderman NB, Jones KJ (1989) Testosterone-induced acceleration of recovery from facial paralysis following crush axotomy of the facial nerve in male hamsters. *Exp Neurol* 105:80–85
- Labroo P, Ho S, Sant H, Shea J, Gale BK, Agarwal J (2016) Controlled delivery of FK506 to improve nerve regeneration. *Shock* 46:154–159
- Labroo P, Shea J, Sant H, Gale B, Agarwal J (2017) Effect of combining FK506 and neurotrophins on neurite branching and elongation. *Muscle Nerve* 55:570–581
- Lee M, Doolabh VB, Mackinnon SE, Jost S (2000) FK506 promotes functional recovery in crushed rat sciatic nerve. *Muscle Nerve* 23:633–640
- Lezana JP, Dagan SY, Robinson A, Goldstein RS, Fainzilber M, Bronfman FC, Bronfman M (2016) Axonal PPARgamma promotes neuronal regeneration after injury. *Dev Neurobiol* 76:688–701
- Lie M, Grover M, Whitlon DS (2010) Accelerated neurite growth from spiral ganglion neurons exposed to the Rho kinase inhibitor H-1152. *Neuroscience* 169:855–862
- Liechty WB, Kryscio DR, Slaughter BV, Peppas NA (2010) Polymers for drug delivery systems. *Annu Rev Chem Biomol Eng* 1:149–173

- Lingor P, Teusch N, Schwarz K, Mueller R, Mack H, Bahr M, Mueller BK (2007) Inhibition of Rho kinase (ROCK) increases neurite outgrowth on chondroitin sulphate proteoglycan in vitro and axonal regeneration in the adult optic nerve in vivo. *J Neurochem* 103:181–189
- Ma TC, Willis DE (2015) What makes a RAG regeneration associated? *Front Mol Neurosci* 8:43
- Madura T, Yamashita T, Kubo T, Fujitani M, Hosokawa K, Tohyama M (2004) Activation of Rho in the injured axons following spinal cord injury. *EMBO Rep* 5:412–417
- Madura T, Kubo T, Tanag M, Matsuda K, Tomita K, Yano K, Hosokawa K (2007) The Rho-associated kinase inhibitor fasudil hydrochloride enhances neural regeneration after axotomy in the peripheral nervous system. *Plast Reconstr Surg* 119:526–535
- Madura T, Tomita K, Terenghi G (2011) Ibuprofen improves functional outcome after axotomy and immediate repair in the peripheral nervous system. *J Plast Reconstr Aesthet Surg* 64:1641–1646
- Manaenko A, Fathali N, Chen H, Suzuki H, Williams S, Zhang JH, Tang J (2010) Heat shock protein 70 upregulation by geldanamycin reduces brain injury in a mouse model of intracerebral hemorrhage. *Neurochem Int* 57:844–850
- Marin J, Blanco T, Marin JJ, Moreno A, Martitegui E, Aragues JC (2019) Integrating a gait analysis test in hospital rehabilitation: a service design approach. *PLoS One* 14:e0224409
- Martin G, Duez H, Blanquart C, Berezowski V, Poulain P, Fruchart JC, Najib-Fruchart J, Glineur C, Staels B (2001) Statin-induced inhibition of the Rho-signaling pathway activates PPARalpha and induces HDL apoA-I. *J Clin Invest* 107:1423–1432
- Martinez FO, Gordon S (2014) The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep* 6:13
- Matos L, Gouveia AM, Almeida H (2017) Resveratrol attenuates copper-induced senescence by improving cellular proteostasis. *Oxidative Med Cell Longev* 2017:3793817
- Mattsson P, Janson AM, Aldskogius H, Svensson M (2001) Nimodipine promotes regeneration and functional recovery after intracranial facial nerve crush. *J Comp Neurol* 437:106–117
- Mattsson P, Frostell A, Bjorck G, Persson JKE, Hakim R, Zedenius J, Svensson M (2018) Recovery of voice after reconstruction of the recurrent laryngeal nerve and adjuvant nimodipine. *World J Surg* 42:632–638
- Mekaj AY, Morina AA, Bytyqi CI, Mekaj YH, Duci SB (2014) Application of topical pharmacological agents at the site of peripheral nerve injury and methods used for evaluating the success of the regenerative process. *J Orthop Surg Res* 9:94
- Mohammadi R, Hirsae MA, Amini K (2013) Improvement of functional recovery of transected peripheral nerve by means of artery grafts filled with diclofenac. *Int J Surg* 11:259–264
- Mokarram N, Merchant A, Mukhatyar V, Patel G, Bellamkonda RV (2012) Effect of modulating macrophage phenotype on peripheral nerve repair. *Biomaterials* 33:8793–8801
- Moschou M, Kosmidis EK, Kaloyianni M, Geronikaki A, Dabarakis N, Theophilidis G (2008) In vitro assessment of the neurotoxic and neuroprotective effects of N-acetyl-L-cysteine (NAC) on the rat sciatic nerve fibers. *Toxicol In Vitro* 22:267–274
- Mueller BK, Mack H, Teusch N (2005) Rho kinase, a promising drug target for neurological disorders. *Nat Rev Drug Discov* 4:387–398
- Mulder T, Nienhuis B, Pauwels J (1998) Clinical gait analysis in a rehabilitation context: some controversial issues. *Clin Rehabil* 12:99–106
- Navarro X (2016) Functional evaluation of peripheral nerve regeneration and target reinnervation in animal models: a critical overview. *Eur J Neurosci* 43:271–286
- Nishio Y, Koda M, Kitajo K, Seto M, Hata K, Taniguchi J, Moriya H, Fujitani M, Kubo T, Yamashita T (2006) Delayed treatment with Rho-kinase inhibitor does not enhance axonal regeneration or functional recovery after spinal cord injury in rats. *Exp Neurol* 200:392–397
- Nunez G, Del Peso L (1998) Linking extracellular survival signals and the apoptotic machinery. *Curr Opin Neurobiol* 8:613–618
- Olson MF (2008) Applications for ROCK kinase inhibition. *Curr Opin Cell Biol* 20:242–248
- Padhy BM, Gupta YK (2011) Drug repositioning: re-investigating existing drugs for new therapeutic indications. *J Postgrad Med* 57:153–160

- Pang X, Yi T, Yi Z, Cho SG, Qu W, Pinkaew D, Fujise K, Liu M (2009) Morelloflavone, a biflavonoid, inhibits tumor angiogenesis by targeting rho GTPases and extracellular signal-regulated kinase signaling pathways. *Cancer Res* 69:518–525
- Pearse DD, Pereira FC, Marcillo AE, Bates ML, Berrocal YA, Filbin MT, Bunge MB (2004) cAMP and Schwann cells promote axonal growth and functional recovery after spinal cord injury. *Nat Med* 10:610–616
- Phan DQ, Schuind F (2012) Tolerance and effects of FK506 (tacrolimus) on nerve regeneration: a pilot study. *J Hand Surg Eur Vol* 37:537–543
- Pillai O, Panchagnula R (2001) Polymers in drug delivery. *Curr Opin Chem Biol* 5:447–451
- Pittier R, Sauthier F, Hubbell JA, Hall H (2005) Neurite extension and in vitro myelination within three-dimensional modified fibrin matrices. *J Neurobiol* 63:1–14
- Poppler LH, Ee X, Schellhardt L, Hoben GM, Pan D, Hunter DA, Yan Y, Moore AM, Snyder-Warwick AK, Stewart SA, Mackinnon SE, Wood MD (2016) Axonal growth arrests after an increased accumulation of Schwann cells expressing senescence markers and stromal cells in acellular nerve allografts. *Tissue Eng Part A* 22:949–961
- Puhl AC, Milton FA, Cvorio A, Sieglaff DH, Campos JC, Bernardes A, Filgueira CS, Lindemann JL, Deng T, Neves FA, Polikarpov I, Webb P (2015) Mechanisms of peroxisome proliferator activated receptor gamma regulation by non-steroidal anti-inflammatory drugs. *Nucl Recept Signal* 13:e004
- Quick TJ, Singh AK, Fox M, Sinisi M, Macquillan A (2016) A quantitative assessment of the functional recovery of flexion of the elbow after nerve transfer in patients with a brachial plexus injury. *Bone Joint J* 98-B:1517–1520
- Quintanilla RA, Utreras E, Cabezas-Opazo FA (2014) Role of PPAR gamma in the differentiation and function of neurons. *PPAR Res* 2014:768594
- Raff MC, Hornby-Smith A, Brookes JP (1978) Cyclic AMP as a mitogenic signal for cultured rat Schwann cells. *Nature* 273:672–673
- Rayner M, Laranjeira S, Evans R, Shipley R, Healy J, Phillips J (2018) Developing an in vitro model to screen drugs for nerve regeneration. *Anat Rec* 301:1628–1637
- Rayner M, Brown H, Wilcox M, Phillips J, Quick T (2019) Quantifying regeneration in patients following peripheral nerve injury. *J Plast Reconstr Aesthet Surg* 73:201–208
- Reid AJ, Shawcross SG, Hamilton AE, Wiberg M, Terenghi G (2009) N-acetylcysteine alters apoptotic gene expression in axotomised primary sensory afferent subpopulations. *Neurosci Res* 65:148–155
- Roloff F, Scheiblich H, Dewitz C, Dempewolf S, Stern M, Bicker G (2015) Enhanced neurite outgrowth of human model (NT2) neurons by small-molecule inhibitors of Rho/ROCK signaling. *PLoS One* 10:e0118536
- Rustemeyer J, Van De Wal R, Keipert C, Dicke U (2010) Administration of low-dose FK 506 accelerates histomorphometric regeneration and functional outcomes after allograft nerve repair in a rat model. *J Craniomaxillofac Surg* 38:134–140
- Saheb-Al-Zamani M, Yan Y, Farber SJ, Hunter DA, Newton P, Wood MD, Stewart SA, Johnson PJ, Mackinnon SE (2013) Limited regeneration in long acellular nerve allografts is associated with increased Schwann cell senescence. *Exp Neurol* 247:165–177
- Scheller K, Scheller C (2012) Nimodipine promotes regeneration of peripheral facial nerve function after traumatic injury following maxillofacial surgery: an off label pilot-study. *J Cranio-Maxillofac Surg* 40:427–434
- Schmandke A, Schmandke A, Strittmatter SM (2007) ROCK and Rho: biochemistry and neuronal functions of Rho-associated protein kinases. *Neuroscientist* 13:454–469
- Schmitt AB, Breuer S, Liman J, Buss A, Schlangen C, Pech K, Hol EM, Brook GA, Noth J, Schwaiger FW (2003) Identification of regeneration-associated genes after central and peripheral nerve injury in the adult rat. *BMC Neurosci* 4:8
- Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L, Finnerty CC, Lopez CM, Honari S, Moore EE, Minei JP, Cuschieri J, Bankey PE, Johnson JL, Sperry J, Nathens AB, Billiar TR, West MA, Jeschke MG, Klein MB, Gamelli RL, Gibran NS, Brownstein BH, Miller-Graziano C,

- Calvano SE, Mason PH, Cobb JP, Rahme LG, Lowry SF, Maier RV, Moldawer LL, Herndon DN, Davis RW, Xiao W, Tompkins RG, Inflammation & Host Response to Injury, L. S. C. R. P (2013) Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci USA* 110:3507–3512
- Sharma N, Coughlin L, Porter RG, Tanzer L, Wurster RD, Marzo SJ, Jones KJ, Foecking EM (2009) Effects of electrical stimulation and gonadal steroids on rat facial nerve regenerative properties. *Restor Neurol Neurosci* 27:633–644
- Stefani M, Markus MA, Lin RC, Pinese M, Dawes IW, Morris BJ (2007) The effect of resveratrol on a cell model of human aging. *Ann N Y Acad Sci* 1114:407–418
- Stewart HJ, Eccleston PA, Jessen KR, Mirsky R (1991) Interaction between cAMP elevation, identified growth factors, and serum components in regulating Schwann cell growth. *J Neurosci Res* 30:346–352
- Subramanian A, Krishnan UM, Sethuraman S (2009) Development of biomaterial scaffold for nerve tissue engineering: biomaterial mediated neural regeneration. *J Biomed Sci* 16:108
- Suckling K (2008) Animal research: too much faith in models clouds judgement. *Nature* 455:460
- Sulaiman OA, Voda J, Gold BG, Gordon T (2002) Fk506 increases peripheral nerve regeneration after chronic axotomy but not after chronic schwann cell denervation. *Exp Neurol* 175:127–137
- Sun HH, Saheb-Al-Zamani M, Yan Y, Hunter DA, Mackinnon SE, Johnson PJ (2012) Geldanamycin accelerated peripheral nerve regeneration in comparison to FK-506 in vivo. *Neuroscience* 223:114–123
- Sundem L, Chris Tseng KC, Li H, Ketz J, Noble M, Elfars J (2016) Erythropoietin enhanced recovery after traumatic nerve injury: myelination and localized effects. *J Hand Surg Am* 41:999–1010
- Tajdaran K, Shoichet MS, Gordon T, Borschel GH (2015) A novel polymeric drug delivery system for localized and sustained release of tacrolimus (FK506). *Biotechnol Bioeng* 112:1948–1953
- Tajdaran K, Chan K, Shoichet MS, Gordon T, Borschel GH (2019) Local delivery of FK506 to injured peripheral nerve enhances axon regeneration after surgical nerve repair in rats. *Acta Biomater* 96:211–221
- Tang BL (2003) Inhibitors of neuronal regeneration: mediators and signaling mechanisms. *Neurochem Int* 42:189–203
- Tang YD, Zheng XS, Ying TT, Yuan Y, Li ST (2015) Nimodipine-mediated re-myelination after facial nerve crush injury in rats. *J Clin Neurosci* 22:1661–1668
- Tedeschi A (2011) Tuning the orchestra: transcriptional pathways controlling axon regeneration. *Front Mol Neurosci* 4:60
- Tetzlaff J, Tanzer L, Jones KJ (2007) Exogenous androgen treatment delays the stress response following hamster facial nerve injury. *J Neuroendocrinol* 19:383–389
- Tos P, Ronchi G, Papalia I, Sallen V, Legagneux J, Geuna S, Giacobini-Robecchi MG (2009) Chapter 4: methods and protocols in peripheral nerve regeneration experimental research: part I-experimental models. *Int Rev Neurobiol* 87:47–79
- Tseng KC, Li H, Clark A, Sundem L, Zuscik M, Noble M, Elfars J (2016) 4-Aminopyridine promotes functional recovery and remyelination in acute peripheral nerve injury. *EMBO Mol Med* 8:1409–1420
- Udina E, Ladak A, Furey M, Brushart T, Tyreman N, Gordon T (2010) Rolipram-induced elevation of cAMP or chondroitinase ABC breakdown of inhibitory proteoglycans in the extracellular matrix promotes peripheral nerve regeneration. *Exp Neurol* 223:143–152
- Van De Velde S, Van Bergen T, Sijnave D, Hollanders K, Castermans K, Defert O, Leysen D, Vandewalle E, Moons L, Stalmans I (2014) AMA0076, a novel, locally acting Rho kinase inhibitor, potentially lowers intraocular pressure in New Zealand white rabbits with minimal hyperemia. *Invest Ophthalmol Vis Sci* 55:1006–1016
- Varejao AS, Cabrita AM, Meek MF, Bulas-Cruz J, Melo-Pinto P, Raimondo S, Geuna S, Giacobini-Robecchi MG (2004) Functional and morphological assessment of a standardized rat sciatic nerve crush injury with a non-serrated clamp. *J Neurotrauma* 21:1652–1670

- Wakino S, Hayashi K, Kanda T, Tatematsu S, Homma K, Yoshioka K, Takamatsu I, Saruta T (2004) Peroxisome proliferator-activated receptor gamma ligands inhibit Rho/Rho kinase pathway by inducing protein tyrosine phosphatase SHP-2. *Circ Res* 95:e45–e55
- Wang L, Yuan D, Zhang D, Zhang W, Liu C, Cheng H, Song Y, Tan Q (2015) Ginsenoside re promotes nerve regeneration by facilitating the proliferation, differentiation and migration of Schwann cells via the ERK- and JNK-dependent pathway in rat model of sciatic nerve crush injury. *Cell Mol Neurobiol* 35:827–840
- Welin D, Novikova LN, Wiberg M, Kellerth JO, Novikov LN (2009) Effects of N-acetyl-cysteine on the survival and regeneration of sural sensory neurons in adult rats. *Brain Res* 1287:58–66
- Wilson AD, Hart A, Brannstrom T, Wiberg M, Terenghi G (2003) Primary sensory neuronal rescue with systemic acetyl-L-carnitine following peripheral axotomy. A dose-response analysis. *Br J Plast Surg* 56:732–739
- Wilson AD, Hart A, Brannstrom T, Wiberg M, Terenghi G (2007) Delayed acetyl-L-carnitine administration and its effect on sensory neuronal rescue after peripheral nerve injury. *J Plast Reconstr Aesthet Surg* 60:114–118
- Wilson AD, Hart A, Wiberg M, Terenghi G (2010) Acetyl-L-carnitine increases nerve regeneration and target organ reinnervation – a morphological study. *J Plast Reconstr Aesthet Surg* 63:1186–1195
- Wood MD, Mackinnon SE (2015) Pathways regulating modality-specific axonal regeneration in peripheral nerve. *Exp Neurol* 265:171–175
- Xiao WD, Yu AX, Liu DL (2014) Fasudil hydrochloride could promote axonal growth through inhibiting the activity of ROCK. *Int J Clin Exp Pathol* 7:5564–5568
- Yan CY, Greene LA (1998) Prevention of PC12 cell death by N-acetylcysteine requires activation of the Ras pathway. *J Neurosci* 18:4042–4049
- Yan Y, Sun HH, Hunter DA, Mackinnon SE, Johnson PJ (2012) Efficacy of short-term FK506 administration on accelerating nerve regeneration. *Neurorehabil Neural Repair* 26:570–580
- Yang WW, Pierstorff E (2012) Reservoir-based polymer drug delivery systems. *J Lab Autom* 17:50–58
- Yin ZS, Zhang H, Bo W, Gao W (2010) Erythropoietin promotes functional recovery and enhances nerve regeneration after peripheral nerve injury in rats. *AJNR Am J Neuroradiol* 31:509–515
- Yuan H, Zhang J, Liu H, Li Z (2013) The protective effects of resveratrol on Schwann cells with toxicity induced by ethanol in vitro. *Neurochem Int* 63:146–153
- Zanon RG, Cartarozzi LP, Victorio SC, Moraes JC, Morari J, Velloso LA, Oliveira AL (2010) Interferon (IFN) beta treatment induces major histocompatibility complex (MHC) class I expression in the spinal cord and enhances axonal growth and motor function recovery following sciatic nerve crush in mice. *Neuropathol Appl Neurobiol* 36:515–534
- Zhang CG, Welin D, Novikov L, Kellerth JO, Wiberg M, Hart AM (2005) Motoneuron protection by N-acetyl-cysteine after ventral root avulsion and ventral rhizotomy. *Br J Plast Surg* 58:765–773
- Zuo J, Neubauer D, Graham J, Krekoski CA, Ferguson TA, Muir D (2002) Regeneration of axons after nerve transection repair is enhanced by degradation of chondroitin sulfate proteoglycan. *Exp Neurol* 176:221–228

Part V

Clinical Aspects

Surgical Techniques in Nerve Repair

Robert Schmidhammer, Rudolf Rosenauer, and Thomas Hausner

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Abstract

This chapter focuses on surgical strategies in nerve repair. Surgical treatment should be adapted to the type and proximity of the peripheral nerve lesion and the time interval since trauma. The gold standard for bridging a segmental nerve defect gap is still autologous interfascicular nerve grafting according to Millesi. However, sources of autologous nerve grafts are limited and therefore the use of nerve allografts and nerve conduits is discussed. If there is a loss of the proximal nerve stump and/or the calculated time interval for successful reinnervation seems to be too long, nerve transfers and end-to-side coaptation are possible treatment approaches. The main goal of our treatment is to provide a maximum of possible nerve fibers for reinnervation. This concept implies that peripheral nerve fiber transfer as the single source for reinnervation is not adequate. If possible, the nerve lesion must be explored and the reconstruction of the lesion should be part of the treatment. Muscle-tendon transfer after nerve reconstruction is an essential part of surgical strategy. Neurolysis is a procedure to decompress nerve fascicles after external compression and neural fibrotic changes. If this procedure is performed in a methodical way and the decompression of fascicles is the main goal, it is a useful therapeutic tool for all nerve compression syndromes. The importance of the gliding tissues around the nerve and their reconstruction in this type of pathology is comprehensively discussed. Finally, some basic strategies are provided for different types of brachial plexus injury, including functional free muscle transfer.

1 Introduction

Surgery of peripheral nerves includes primary or secondary reconstruction of an injury to a branch of the peripheral nervous system. This comprises the treatment of complete or partial nerve transections, the reconstruction of a simple nerve defect or the more complex nerve plexus, as well as the treatment of neural pain conditions like painful neuroma formation or compression syndromes. Nerve transections are typically treated by epineural repair. In segmental nerve defects, the gold standard of nerve reconstruction remains autologous interfascicular nerve grafting as according to Millesi (Millesi et al. 1972b). After a publication by Oberlin in 1994, peripheral nerve fiber transfer procedures have been added to proximal nerve fiber transfers (e.g., accessory nerve to suprascapular nerve transfer). Treatment possibilities have been significantly expanded in recent years through the increased use of peripheral nerve transfers and the introduction of new products such as allografts or conduits. The treatment of nerve pain or compression syndromes includes surgical therapy of painful neuroma formation, decompression, and microsurgical neurolysis, as well as the restoration of the gliding capacity of peripheral nerves. The outcome of a lesion to the peripheral nervous systems depends to a large extent on factors beyond any therapeutic influence, like type of injury, proximity of nerve lesion, apoptosis, and age. Surgical treatment strategy, pre- and postoperative rehabilitation of nerve injuries, and also patient compliance influence functional outcome.

2 Basics of Nerve and Muscle Regeneration

If a peripheral nerve loses contact with the central nervous system or the cell nucleus and thus the input of efferent signals, Wallerian degeneration of the peripheral nerve stump occurs within a few days. In 1850, Waller described coagulation or curdling of the myelin within 5 to 6 days after transection of the nerve, fragmentation and partial absorption within 7 to 9 days, and the appearance of an amorphous granular structure afterwards (Waller 1850). As a result, the axon and myelin are completely degraded by migrating macrophages. This is the basis for the increased expression of growth factors, which should promote axonal regeneration (Stoll and Müller 1999; Stoll et al. 2002; Ehlers 2004).

After coaptation of two nerve endings, the microscopic gap between the stumps is first filled with serum and within a few days with fibrin and then macrophages (Liu 1992; Zhao et al. 1992; Dahlin et al. 1995). The fibrin is converted into collagen, and Schwann cells migrate. Subsequently, axon sprouting starts in conjunction with other Schwann cells (Williams et al. 1983; Longo et al. 1984; Liu 1992). After the formation of a growth cone, axon growth progresses at a rate of about 1 to 2 mm per day (Tinel 1916).

The structural and functional unit of a muscle is the sarcomere which is powered by the interaction of overlapping myosin and actin filaments. Thick myosin filaments are anchored to the M line and thin actin filaments are anchored to the Z line. An increase of the overlapping of myosin and actin results in muscle contraction. Sarcomeres vary in length between 1.5 and 3.5 micrometers, depending on their state of contraction. Thousands of sarcomeres are attached in series to form a myofibril and hundreds of myofibrils are linked to form a muscle cell. A muscle contraction is initiated by the release of calcium which binds to tropomyosin, changing its configuration and started by the change in configuration of the myosin head, advancing the thin filament, drawing the M and Z lines closer together (Craig and Padrón 2004). The motor unit is the elemental circuit of motor control. It consists of motoneuron and the muscle fibers it serves. Motor units are classified as fast-twitch fatigable (FF); fast-twitch intermediate (Fint); fast-twitch fatigue resistant (FR); and slow (S). During forceful muscle contraction, motor units are recruited according to their size, starting with small size S units and ending with the largest type FF units. Muscle fiber types are determined by the identity of the myosin heavy chain. Type IIb is found in FF fibers, type IIa in FR fibers, and type I in S fibers. Metabolic capabilities of type IIb fibers and, to a lesser extent, type IIa fibers are more glycolytic, specialized to run anaerobically. Type I in S fibers store less glycogen with higher oxidative metabolism. Type IIa fibers of FR units contain both aerobic and anaerobic enzyme systems. Therefore, they are able to contract rapidly and can resist fatigue during a longer period of activation. S fibers and some FR fibers are recruited for standing. FR fibers are mostly active during walking and FF fibers are activated during running and jumping. This is also the recruitment sequence of muscle fibers. The force of a muscle depends not only on the identity of the motor unit firing, but also on the frequency of discharge. To

establish low forces, motoneurons begin firing at 5–10 Hz, meaning 5–10 contractions per second. In response to incremental demands the firing rate can be increased to 20–40 Hz (Enoka and Fuglevand 2001). Instantaneous production of high forces begins with a period of contraction at 50–60 Hz followed by a rapid decline in discharge frequency. In some humans lower extremity muscles and fast and slow motor units have similar minimum and maximum firing rates. When the muscle is activated repetitively, force does not increase as a linear function of firing rate (Burke and Barnes 2006). For example, activating the FR motor unit in the cat from the first impulse (65 ms) to the second impulse (10 ms) will double the force. This doublet effect is also seen in humans (Thomas et al. 1999) and may serve to diminish the consequence of fatigue (Bigland-Ritchie et al. 2000).

After nerve injury and regeneration, muscle performance is influenced by the number of dorsal root ganglion (DRG), neurons, and their central connections that have reinnervated the muscle sensory organs. After nerve repair many muscle receptors are left without reinnervation or are innervated by inappropriate nerve fibers. In a reinnervated motor nerve only 50–75% of muscle spindles and Golgi tendon organs are reinnervated. Many respond pathologically to stretch. A variety of abnormal innervation patterns is the result of a lack of reinnervation specificity. The properties of denervated muscle fibers are respecified by the activity level of the reinnervating motoneurons. This was first demonstrated by cross-reinnervation of a fast FDL muscle and a slow soleus muscle. The FDL muscle became a slowly contracting muscle and the soleus a fast contracting muscle (Windisch et al. 1998; Ralston et al. 2001).

Motoneuron loss in adults after peripheral nerve transection is minimal compared to neonatal nerve injury and proximal nerve lesions (Ma et al. 2003). The force of a muscle fiber is generated by motor units and gradually becomes proportional to the size of its new motoneuron. Therefore, the surgical strategy in nerve repair should be to provide a maximum of possible motor reinnervation power to the target organs and thus improve muscle strength and muscle endurance.

3 Surgical Treatment According to the Type of Peripheral Nerve Lesion

Depending on the type of nerve injury, there are numerous different techniques available to achieve reinnervation of the affected motor and sensory organs. In general, the recovery of motor function is given a much higher priority. The choice of technique is based on the mechanism of the accident, the number and types of end organs to be reinnervated, the presence of axon donors, the location of the lesion, and the time since the accident. After considering these factors, the appropriate technique can be selected (see Fig. 1). Often a combination of techniques is necessary. It should also be mentioned that the surgical therapy of nerve injury is only a part of the overall treatment (see also chapter ► [“Rehabilitation of Nerve Injuries”](#)).

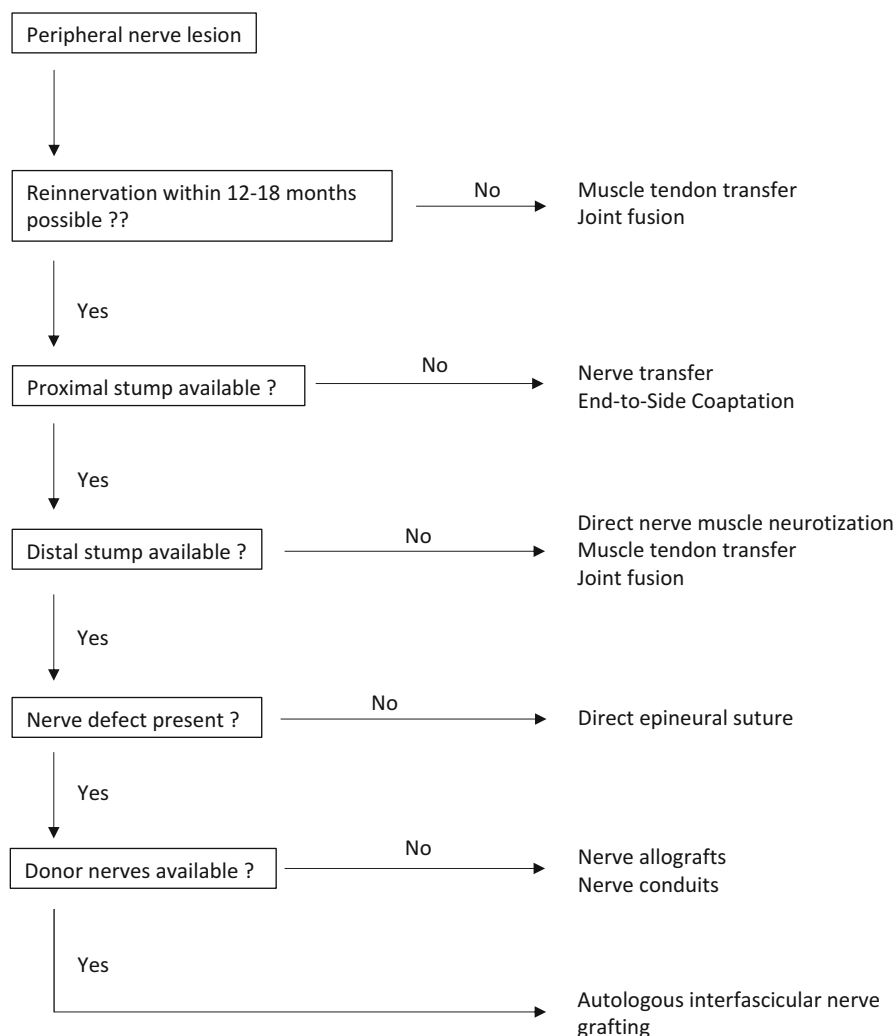


Fig. 1 Peripheral nerves are reconstructed depending on the type of the lesion. Preoperatively, the injury and possible axon donors have to be evaluated to guarantee sufficient reinnervation

3.1 Partial or Complete Transection of a Peripheral Nerve

A partial or complete transection of a peripheral nerve is usually treated using a direct epineurial suture with a monofilament 8–0 or smaller suture. Interfascicular suturing is no longer recommended due to the increased grade of fibrosis. Alternatively, gluing is possible if small nerves are injured. In the case of fresh stab or cut injuries, this can be done immediately. The establishment of absolute tension-free coaptation is necessary (Millesi et al. 1972a; Millesi 1973),

otherwise the intraneural blood supply is impaired and the risk of scarring of the nerve ends is increased (Clark et al. 1992). In case of heavily contaminated wounds, open wound treatment can be carried out initially and, in the absence of infection, secondary suturing can be performed until about 7 days after the injury. However, if the injury is a combination which also involves a contusion of the nerve, secondary reconstruction is recommended. The same applies in the presence of a nerve defect with no possibility of tension-free coaptation of the nerve endings (see Sect. 3.2).

3.2 Nerve Defects

Segmental nerve defects can be caused by the accident itself or by secondary fibrosis of the nerve endings after a contusion. Furthermore, a certain retraction and fibrosis of the nerve endings also occurs after stab or cut injuries with late reconstruction. After dissecting the nerve endings, a defect can also occur which cannot be bridged by a direct suture without tension.

3.2.1 Interfascicular Autologous Nerve Grafting

The gold standard for treating a nerve defect is interfascicular autologous nerve grafting, as introduced by Hanno Millesi (Millesi et al. 1967, 1972a; Millesi 1985a). Despite Waller's degeneration of the axons within the nerve graft, the transplantation of the nerve skeleton and Schwann cells lays the foundation for regeneration. Approximately 4 weeks after reconstruction, the axons sprout from the proximal nerve stump and grow into the transplant.

In general, sensory nerves are used for transplantation, as these are associated with an acceptable donor site morbidity. The use of motor nerves does not lead to any regenerative advantage with regards to motor function due to the complete degeneration of the axons contained in them. The direction of implantation is also not associated with any changes in the prognosis.

Possible Donor Nerves

Sural Nerve

For nerve defects larger than 5cm, the sural nerve is the absolute work horse of nerve grafting. Depending on the length of the lower limb, grafts of up to 40cm can be harvested through small incisions. The proximal subfascial location means that painful neuromas rarely develop if the stump is adequately treated. Furthermore, harvesting morbidity with hyposensitivity at the lateral area of the foot is rather low, being seen in 50% of patients.

Saphenous Nerve

The saphenous nerve is the only real alternative to the sural nerve if long nerve grafts are needed. Disadvantages are the inconsistent thickness and increased soft tissue trauma when harvesting it.

Medial Antebrachial Cutaneous Nerve

The medial antebrachial cutaneous nerve runs subfascially along the medial upper arm. The full length can be harvested. Donor site morbidity can be reduced by end-to-side coaptation of the distal stump to the median nerve (Poppler et al. 2015).

Other Nerves

For smaller nerve defects, the anterior branch of the medial antebrachial cutaneous nerve, superficial radial nerve, and the dorsal branch of the ulnar nerve are available. To reconstruct defects of the finger nerves, the interosseus posterior nerve can also be used. However, the interosseus posterior nerve has a high amount of fibrotic tissue and few neurons (Higgins et al. 2002).

3.2.2 Nerve Allografts

The main disadvantages of autologous transplants are limited availability and, to some extent, donor site morbidity. Processed nerve allografts are human nerves from which antigenic or cellular structures of the donor have been removed while the scaffold remains intact. An immunosuppressive therapy is no longer necessary (Siemionow and Sonmez 2007). The reconstruction of the nerve defect works in a manner analogous to autologous grafting. According to the current data, indications are mainly in short nerve gaps of a few centimeters or in the treatment of painful neuroma, especially if autologous transplants are no longer available or usable. The motor and sensory regeneration of longer nerve gaps remains insufficient (Brooks et al. 2012; Cho et al. 2012).

3.2.3 Nerve Conduits

A nerve conduit is a tube of nondegradable or degradable material that provides a guiding structure for growing axons to reach their target organs. High flexibility and high permeability are important to ensure adequate nutrition of the growing axons. At the same time, the direction of axon growth must be maintained and the loss of axons through outgrowth outside of the conduit should be kept to a minimum. The biggest advantage of using a nerve conduit is the absence of donor site morbidity, similar to allografts.

a) Natural, Biodegradable Nerve Conduits

Small nerve defects of a few millimeters can be bridged by vessels filled with muscle. Advantages are the high compatibility and the high diffusion capacity. The musculature within the vein prevents collapse and facilitates Schwann cell migration. Major indications are the reconstruction of shorter nerve defects in more distal lesions. A special application is in acute care under regional plexus anesthesia for fresh wounds. In these cases, the vein and the musculature can be harvested next to the wound and the possibility of secondary reconstruction in the absence of regeneration remains preserved (Tos et al. 2007, 2012).

b) Natural, Nonbiodegradable Nerve Conduits

Natural, nondegradable nerve conduits, such as chitosan or NeuraGen[®], play a minor role in the reconstruction of nerve defects. Individual animal studies show promising results for

short nerve defects up to 1cm. According to the literature, there is no wider application in humans (Wang et al. 2012; Bāk et al. 2017; Yin et al. 2018).

c) Synthetic Nonbiodegradable Nerve Conduits

Synthetic, nonbiodegradable nerve conduits bear the risk of scarring, infections, chronic inflammation, and thereby nerve compression (Kakinoki et al. 1995). According to the literature, the risk of necessary secondary removal of the conduit is as high as 50%. Therefore, the use of such tubes is not recommended (Lundborg et al. 1997, 2004).

d) Synthetic Biodegradable Materials

Synthetic, biodegradable conduits such as Neurotube[®] or Neurolac[®] show promising results in bridging small nerve defects up to 2cm (Kehoe et al. 2012). Although there is no risk of chronic inflammation in these cases, an increased rate of reoperation is described in the literature (Bertleff et al. 2005).

3.3 Loss of the Proximal Nerve Stump

Loss of the proximal nerve stump occurs only in the event of root avulsion and most frequently in lesions to the brachial plexus. In these cases, the axons required for regeneration must be made available from other nerves or even the other side of the body. Various techniques are described for these indications.

The greatest risk of peripheral nerve transfers and end-to-side coaptation of individual nerve fibers is seen in the failure to reconstruct the primary lesion. In the authors' opinion, the reconstruction of the actual lesion should be the basis of all treatment. Even if this alone often does not lead to sufficient motor and sensor recovery, it should be done to achieve the maximum possible regeneration from the available proximal axon donors and for the possible therapy of deafferentation pain, for example, in the case of a root avulsion.

3.3.1 Nerve Transfers

In a nerve transfer, individual fascicles or, rarely, the entire nerve are dissected and thereafter coapted directly or using a nerve graft onto another nerve, a nerve branch, or a motor branch in end-to-end fashion. A distinction can be made between proximal and distal nerve transfers, whereby the term refers to the proximity of the coaptation site to the target organ, in most cases the muscle.

Proximal Nerve Transfers

Transferring the phrenic nerve, spinal accessory nerve, or intercostal nerves represent proximal nerve transfers (de Mendonça et al. 2020). These transfers are mainly used when treating brachial plexus injuries. Compared to distal transfers, axons are not provided as specifically. The phrenic nerve is mostly used to restore elbow flexion by transferring it to the musculocutaneous nerve. This procedure was

first described by Lurje, Gu, and Ma (Lurje 1948; Gu and Ma 1996; Flores and Socolovsky 2016). The spinal accessory nerve is mostly transferred to the supra-scapular nerve to restore shoulder abduction and external rotation as effectively as possible (Segal et al. 2019). Bertelli et al. restored abduction of 60° in 110 patients (Bertelli and Ghizoni 2016). Intercostal nerves are used if axon donors are highly limited. Due to the fact that they are mixed nerves, results are highly inconsistent (de Mendonça et al. 2020).

Contralateral C7 Transfer

A special technique of proximal nerve transfers is the contralateral C7 transfer. This technique was introduced by Gu in 1986 (Gu 1989). It is mainly used for severe brachial plexus lesions with multiple root avulsions and highly limited available axon donors. In this technique, the anterior and posterior division of the root C7 is dissected and dispensable fascicles innervating, for instance, the pectoralis major and triceps brachii muscle are transferred to the contralateral side using nerve grafts. In theory, C7 innervated muscles are fully cross-innervated by the roots C6 and C8 (Chuang et al. 1993). However, we still do not recommend transferring the whole root C7 to the contralateral side due to the possibility of donor morbidity, especially since in these patients the other limb is impaired by a brachial plexus lesion.

The main downside of this technique is the long distance that axons have to bridge to reach their target organs. In addition, the patients must learn how to control the supplied muscles through extensive physiotherapy. A distinction must be made, however, between whether this technique is used in adult patients due to a traumatic plexus lesion or in newborns due to an obstetric lesion. Both the shorter distance and the better relearning ability enhance results in the latter (Chen et al. 2007; Lin et al. 2011; Leblebicioglu et al. 2016). However, these factors are also the main reasons for the significantly different results reported in the literature (Gu et al. 2002; Gu 2007; Oberlin et al. 2009; Sammer et al. 2012).

Distal Nerve Transfers

The main advantage of this technique is faster reinnervation; the axons usually have to cover a much shorter distance to the target cells. In addition, muscles can be supplied whose innervation is no longer present due to root avulsion. This technique was presented for the first time in 1984 (Narakas 1984a). Nerve transfers gained popularity through publications by Oberlin. Other authors have worked on this topic and Susan Mackinnon was a powerful supporter of peripheral nerve transfers (Oberlin et al. 1994; Mackinnon and Novak 1999).

The most important prerequisite for nerve transfers is an understanding of the topography within a nerve. Microsurgical preparation allows the individual fascicles of a nerve to be visualized and parts that are dispensable can be identified by electrical stimulation. Therefore, motor donor fascicles should be transferred to motor recipient fascicles or even better motor branches. For example, in the double fascicular transfer according to Mackinnon, dispensable motor branches of the wrist flexors are transferred to muscle branches of the brachialis and biceps brachii muscle

(Mackinnon et al. 2005). Due to the good supply of wrist flexors, this function remains preserved and the axons only have to bridge a few centimeters to their target organs in the muscles. This technique is especially used in root avulsions of C5 and C6. However, it can also be used to support earlier reinnervation. It has been shown that, in the case of far proximal lesions, a nerve transfer in addition to the reconstruction of the primary lesion significantly improves the result (Schmidhammer 2019).

Up to now, numerous nerve transfer procedures have been published, especially on techniques used when reconstructing the brachial plexus. In general, the treatment of each lesion has to be chosen individually depending on which axons are available and which function has to be reconstructed (Ray et al. 2015; Domeshek et al. 2019).

3.3.2 End-to-Side Coaptation

The distal nerve stump can be coapted end to side to the healthy axon donor after creating an epineurial window. Opening this window interrupts the antineurotrophic continuity of the wall and promotes a realignment of the Schwann cells (Bontioti and Dahlin 2009). Studies have already shown that the presence of a distal nerve stump has a strong effect on axon growth (Papalia et al. 2016). The creation of an epineurial window of 1 to 5 mm in size makes the axons that have grown into the nerve stump even larger and more myelinated (Kovačič et al. 2012).

While this technique showed promising results in animal experiments, this could only be partially confirmed for motor recovery in humans (Stamatoukou et al. 2006; Schmidhammer et al. 2009; Wang et al. 2011; Yang et al. 2014). Regeneration in far proximal lesions is sometimes insufficient, especially when coaptating mixed nerves. In these cases this is not recommended as a sole reconstruction technique (Pondaag and Gilbert 2008). In more distal lesions, good reinnervation can be achieved by targeted reinnervation of individual muscle groups (Millesi and Schmidhammer 2008; Schmidhammer et al. 2009). A further area of application is the treatment of neuropathic pain due to root avulsion. Acceptable results of sensitivity with significantly improved pain status and unchanged sensitivity in the donor area could be shown (Leechavengvongs et al. 2011).

However, it has been noticed that, in end-to-side coaptation, a loss of function from the donor nerve occurs to some extent. Additionally, it has yet to be clarified which type of donor (agonist, antagonist, or sensory) works best to achieve the desired result (Papalia et al. 2007; Colonna et al. 2015).

A different technique is end-to-side coaptation in supercharge fashion. In this case, another donor nerve is coapted onto the reconstructed nerve distally end to side, so that axons are provided as early as possible. This technique is most useful when treating proximal lesions of the ulnar nerve (Barbour et al. 2012).

3.4 Loss of the Distal Nerve Stump

In rare cases, the distal nerve stump or motor branches are not available or dissectible. This is mainly the case in proximal lesions of the supply of the trapezius

muscle, in severe crush injuries or far distal nerve lesions close to the neuromuscular junction. In all cases, the original anatomical structure is highly destructed. Depending on the availability of axon donors, partial reinnervation can be achieved. However, it should be mentioned that results of such procedures always remain inferior compared to reconstruction of the nerve supply via the primary motor branches.

3.4.1 Direct Nerve-Muscle Neurotization

This technique was originally described by Hacker [1908](#). He achieved partial reinnervation of the trapezius muscle by placing nerves directly into it (Hacker [1908](#)). Although some promising results were reported in animal experiments, this technique remains one of the last options to reinnervate a specific muscle (Park et al. [2000](#); Askar et al. [2001](#)). Millesi modified this technique by guiding innervated motor branches of other muscles to the denervated muscle using a nerve graft (Millesi [1985a](#)). Brunelli and Brunelli showed that by dissecting the axon donor into as many fascicles as possible, a wide reinnervation of the targeted muscle is possible (Brunelli and Brunelli [1980](#)). One of the main factors affecting the result of direct nerve muscle neurotization is the age of the patient. It has been found that the presynaptic neuromuscular junction undergoes degenerative changes during aging. Functional recovery seems to be better in patients at the age of 20 years or younger (Apel et al. [2009](#); Terzis and Karypidis [2009](#)).

3.4.2 Muscle-Tendon Transfer and Joint Fusion

If a reinnervation of the denervated muscles is not possible, the only option to restore function is by transferring existing muscles. By fusing joints (see Sect. [3.5.3](#)), the required muscles can be reduced. The main goal is usually the restoration of the grasping function of the upper limb (see Sect. [3.5.2](#)).

3.5 Late Reconstruction of Far Proximal Nerve Lesions

If a nerve injury is treated at a very late stage (later than 12 months), an essential part of the strategy is to determine the degree of atrophy of the muscles by ultrasound examination. In animal models, a decrease in muscle diameter could be observed from day 14 of denervation. As a consequence, echogenicity within the muscles increases and the muscle-typical texture dissolves (Küllmer et al. [2008](#)). Furthermore, initial fasciculations can be detected (Pillen and van Alfen [2011](#)). In general, regeneration of denervated muscles can be achieved within about 1 year after the accident (Stefancic et al. [2016](#)). If there is still detectable fibrillation, the time of reinnervation can be extended to a maximum of 18 months.

3.5.1 Peripheral Nerve Transfers

A peripheral nerve transfer can be performed as a supportive procedure or as a sole reconstruction depending on the grade of degeneration of the muscles. In general, the technique is similar to that described in Sect. [3.3.1](#). However, especially in late cases

and far proximal lesions, axonal supply should be achieved as fast as possible. Therefore, peripheral nerve transfers are preferred. Additionally, the expected regeneration of axons from the reconstructed proximal lesion is weak due to their late arrival. However, this fact makes the reconstruction more predictable. First, denervated muscles should be detected and the desired function defined. Thereafter, specific nerve transfers can be performed. Care must be taken to keep the distance between the donor and the muscle as short as possible. In nerve transfer, coapting the donor directly to a muscle branch is favorable.

In late or far proximal lesions of the median and ulnar nerve, the intrinsic hand muscles are especially at risk of being reinnervated too late. Therefore, special nerve transfers have been established. Most common and best studied is the transfer of the anterior interosseus nerve to the deep branch of the ulnar nerve. For this transfer, advantages have been described even in acute cases of a high ulnar nerve lesion (Koriet et al. 2020). Sensibility of the ulnar border of the small finger can be achieved by a transfer of branches from the superficial radial nerve to the tenth finger nerve (Gottschalk and Bindra 2012). In the case of a combined proximal lesion of the median and ulnar nerve, even a transfer of supinator branches via the anterior interosseus nerve bridge has been described (Namazi and HajiVandi 2017). In cases of a high median nerve lesion, a transfer of the common digital nerve to the fourth web space, which is supplied by the ulnar nerve, to the radial digital nerve of the index and the ulnar digital nerve of the thumb has been described. Sensibility for pinch grasping can be achieved in this way (Isaacs and Ugwu-Oju 2016). Alternatively, a transfer of branches of the superficial radial nerve can be performed to restore fingertip sensibility (Bertelli and Ghizoni 2011). Numerous transfers have been described to reinnervate the thenar branch in high median nerve lesions (Schultz and Aiache 1972; Wang and Zhu 1997; Vernadakis et al. 2004). Most promising seems to be a transfer of the abductor digiti minimi motor branch to the thenar motor branch (Bertelli et al. 2018).

3.5.2 Muscle-Tendon Transfer

Depending on the nerve lesion and available muscles, numerous muscle-tendon transfers have been described. In general, muscle strength is reduced by one grade when relocating it for another function. However, in most cases, sufficient strength and motion can be achieved. In many cases motor relearning has to be supported to some extent by postoperative physiotherapy, making it an essential component for success.

Shoulder stability can be achieved by trapezius or supraspinatus muscle transfer (Mayer 1927; Bateman 1955; Saha 1967; Schmidhammer 2014). External rotation is augmented by teres major and/or latissimus dorsi transfer (Al-Qattan 2003; Terzis and Kostopoulos 2010). According to our experience, in most cases the teres major muscle provides sufficient strength for external rotation. The latissimus dorsi muscle can be left in place. Elbow flexion can be augmented by a Steindler procedure, whereas elbow extension rarely needs a muscle transfer (Steindler 1919). Multiple muscle-tendon transfers are possible on the forearm and wrist. In the case of a proximal radial nerve lesion, a triple muscle-tendon transfer (pronator teres to

extensor carpi radialis brevis, palmaris longus to extensor pollicis brevis, and flexor carpi radialis to extensor digitorum communis) can be performed with reliable results (Abdullah et al. 2020). Reconstruction of wrist and finger flexors is mainly achieved by transferring radial nerve innervated muscles (Cooney 1988). More challenging is the reconstruction of the opposition and adduction of the thumb, i.e., according to Littler (Littler 1949).

3.5.3 Joint Fusion

Joint fusion can be taken into consideration if few muscles are available. In the upper limb, it is mainly the wrist joint that ought to be fused. Available flexors and extensors of the wrist can then be transferred for different functions. In rare cases and in severe brachial plexus lesions, fusion of the glenohumeral joint is performed. Although fusion of the ankle joint on the lower limb is possible, this should only be considered as a salvage procedure in proximal sciatic nerve lesions.

3.6 Nerve Compression Syndromes

Treating compression syndromes of peripheral nerves is the most common cause for surgery of a peripheral nerve. In most cases there is a simple compression of a peripheral nerve by the surrounding tissue and/or reduced gliding capacity of the peripheral nerve. The reasons for compression or gliding problems are extremely heterogeneous. External decompression can remedy the symptoms.

In principle, every surgical procedure on a peripheral nerve can result in scarring of the nerve with its surrounding tissue, although the risk is very low. The probability increases with the increasing number of surgeries in the same region. As a consequence, the gliding function of the nerve provided by the paraneurium is diminished. It is followed by fibrosis of the epi-, peri-, and later even endoneurium, and in this way increasing compression of the nerve occurs (Millesi et al. 1990). The consequences can be neuropathic pain and possible loss of function. Due to repeated reoperations and the usually long medical record of these patients, this situation is an extraordinary challenge for every treating surgeon.

The degree of compression and the scarring of the peripheral nerve and its surrounding tissue is decisive in defining the appropriate treatment. In many cases this can only be clarified intraoperatively. A stepwise procedure is mandatory when treating such cases (see Fig. 2). The neurolysis procedure must stop immediately when the goal of this treatment, the decompression of fascicles, has been achieved. Informed consent for the different treatment options must be obtained preoperatively.

3.6.1 Simple Nerve Compression Syndromes

Undoubtedly the most frequent one is the carpal tunnel syndrome. Over the years, multiple open, mini-open, or endoscopic approaches have been described. In most cases the surgical treatment is successful with complete regeneration of sensibility and motor function. The main predictor for incomplete regeneration is late surgery

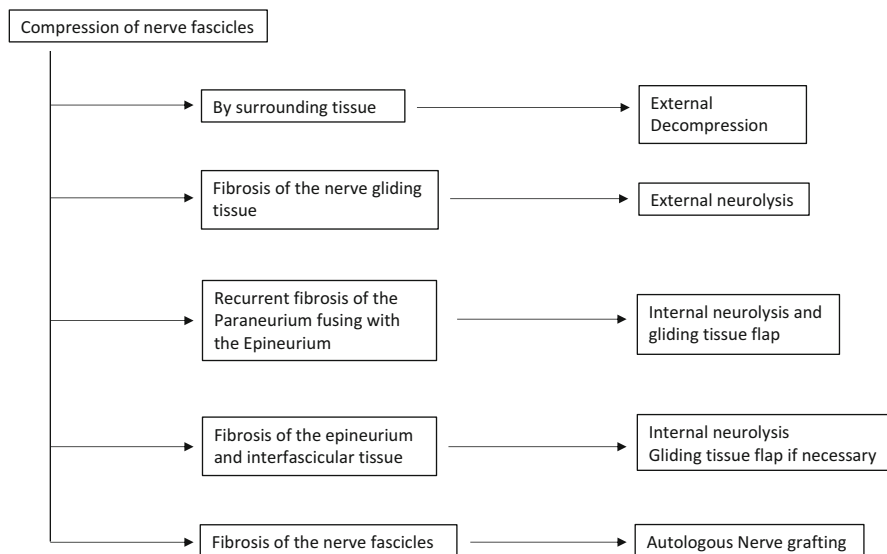


Fig. 2 A microsurgical neurolysis of a peripheral nerve is a stepwise procedure with the aim of decompressing all fascicular structures. All reconstructive procedures must be mastered. If fibrous degeneration of fascicles is found, they must be excised and reconstructed by suturing or autologous grafting

with preoperative atrophy of the thenar muscle. In rare cases, scarring of the nerve occurs postoperatively, leading to a neuropathic pain syndrome. This complication makes an easily treatable carpal tunnel syndrome become a challenging procedure for every surgeon (see Sect. 3.6.2). The second most frequent compression syndrome is the cubital tunnel syndrome. In these cases, luxation of the ulnar nerve over the ulnar epicondyle represents a possible complication. Subcutaneous or sub-muscular transposition of the nerve or removal of the ulnar epicondyle might be necessary.

Other rare compression syndromes exist, both on the upper and lower limb. In most cases, direct surgical decompression of the nerve is performed with satisfactory results. Besides this, the authors would like to highlight the increasing importance of ultrasound imaging for the diagnosis of compression syndromes. Although nerve conduction velocity remains the gold standard for most diagnoses, dynamic imaging holds much potential to exactly localize these nerve compressions. The most important criterion of a nerve compression syndrome is that there is no damage to the nerve itself or its gliding tissue.

3.6.2 Neurolysis

In neurolysis, a peripheral nerve and especially its fascicles are decompressed from external and internal scar tissue. This procedure takes place step by step and as according to Millesi (Millesi et al. 1993; Millesi 1997) (see Fig. 1).

In the course of external neurolysis, the nerve is freed from scarring to the surrounding tissue. The goal is to enable it to move as a unit within the surroundings. It is also important to relieve the nerve of compressible connective tissue. Old scars should not be opened if possible.

If after the external neurolysis a rough hardening of the epineurium is palpable, an internal neurolysis must be performed. Here, too, a step-by-step procedure is performed, and the epineurium is opened first. Afterwards, the degree of fibrosis of the perineurium must be determined empirically by palpation. The nerve is palpated starting in the healthy tissue and compared to the site of the lesion. Hardenings correspond to fibrosis and are usually clearly distinguishable from healthy tissue. If the epineurium is scarred, the individual fascicles must be dissected and decompressed as well.

These stages of fibrosis do not usually affect the entire circumference of a peripheral nerve equally. It is therefore possible that some fascicles are completely fibrosed and others are barely affected.

3.6.3 Direct Epineurial Suture and Nerve Grafting

If a complete fibrosis of individual fascicles exists, these must be reconstructed. The palpation findings, i.e., a hardening of the fascicles, but also a preoperative loss of function, is diagnostically helpful. Furthermore, motor fascicles can be electrically stimulated and evaluated intraoperatively. Often the final decision about the fibrosis of individual fascicles can only be made under the surgical microscope. If confirmed, the affected area must be excised completely. Subsequently, a direct epineurial suture can be performed if this is possible without tension (see Sect. 3.1). If the nerve gap is too large, a nerve grafting procedure must be performed (see Sect. 3.2.1).

3.6.4 Reconstruction of the Gliding Tissue

If there is recurrent fibrosis of the gliding tissue of a nerve and a lack of success of neurolysis, a local or free gliding tissue flap can be transplanted (Millesi et al. 1994). A distinction must be made between true gliding tissue flaps and fatty tissue flaps. The hypothenar fat pad flap is most often used in the treatment of recurrent carpal tunnel syndrome with good success (Strickland et al. 1996; Mathoulin et al. 2000; Craft et al. 2007). Besides this, numerous flaps have been described for this challenging entity (Reisman and Dellon 1983; Rose et al. 1991; Tham et al. 1996). All of these flaps consist of large amounts of tissue which are thought to protect the nerve. This may be beneficial but could also be a source of compression after closing of the wound.

Yamamoto reported satisfying results using a temporoparietal fascial flap for the treatment of recurrent fibrosis of the superficial radial nerve. In this case, this procedure was performed within 5 months after the first neurolysis (Yamamoto et al. 2015).

Coverage of the brachial plexus with gliding tissue flaps has been well documented (Uhlshmid 1978; Brunelli 1980; Narakas 1984b; Brunelli and Brunelli 1985; Millesi 1985b; Lahiji et al. 2017).

One of the main factors of success when treating recurrent fibrosis of peripheral nerves is the duration of symptoms. Unfortunately, this is rarely documented in the literature reporting on gliding tissue flaps to treat recurrent fibrosis. According to the authors' experience, the results of such surgeries are promising if they are performed within 1 year. Afterwards the chance of a complete recovery of the symptoms decreases.

3.7 Painful Neuroma Formation

Neuroma formation arises after a partial or complete transection of a peripheral nerve as an attempt by the proximal stump to regenerate and reconnect to the target organ. Most often neuroma formation is painful if it is located in the subcutaneous tissue. Furthermore, neuroma formation can arise within a peripheral nerve in continuity after stretching it. Therapy is adjusted depending on the pain and the loss of function. The best prophylaxis of recurrent neuroma formation after resection is restoring the continuity of the nerve by direct epineurial suture or nerve grafting. This can also be done with two proximal nerve stumps, for example, when treating painful neuroma formation of digital branches of the median nerve after finger amputation injuries (Belcher and Pandya 2000; Martins et al. 2015). However, there are cases, i.e., neuroma of a far peripheral sensory nerve, in which these approaches are not possible or reasonable. In this case, resecting it and relocating the proximal nerve stump into the subfascial tissue is a viable treatment. Alternatively, relocating the nerve stump into bone has been discussed. Although this technique is not new (Boldrey 1943) recent data is limited. We therefore recommend this technique only in limited indications as a salvage procedure (Goldstein and Sturim 1985). According to the authors' experience, treatment by implantation of long allografts of 10 cm and more is also possible. Although axons seem to stop growing after a few centimeters, this principle has yet to be investigated in depth and remains incompletely understood.

3.8 Reconstruction of the Brachial Plexus

The reconstruction of the brachial plexus is a topic that alone easily fills congresses lasting several days. Therefore, only basic strategies will be discussed here.

First of all, a distinction must be made between obstetric and traumatic plexus lesions. This changes the focus of the therapy considerably. In the case of an obstetric brachial plexus lesion, for example, the focus is on restoring hand function. In the case of a traumatic brachial plexus lesion at an advanced age of the patient, restoration of hand function is not possible if all the roots are injured because of the long distance involved. Here, the function of the shoulder and elbow is prioritized. Furthermore, a distinction must be made between the affected roots and the type of injury.

a) Root Avulsion

A root avulsion is the most serious injury, as the reimplantation of nerve roots into the central nervous system has not been successful (Carlstedt et al. 2000). In these cases, the required axons must be provided by nerve transfers. This can be done locally but also from

contralateral. The decisive factor is to transfer purely motor neurons. Mixed nerves, such as the intercostal nerves, for example, promise only very uncertain success.

b) Root Tear

After neurolysis, a root tear can be addressed by direct epineural suture or nerve grafting. It is important to continue neurolysis and dissection until healthy nerve stumps are presented. Possible scar tissue must be removed.

c) Root Compression

If there is only external or internal compression of the nerve roots by scar tissue, microsurgical neurolysis can be performed stepwise. Preserved conduction of the nerves is confirmed intraoperatively by electric stimulation.

The therapeutic procedure is further planned depending on the affected roots.

a) C5/C6/C7 Lesion

An upper lesion is associated with the relatively best prognosis due to the relatively short path for the axons to their target organs. The reconstruction of the primary lesion should be accompanied by peripheral transfer, e.g., according to Oberlin or Mackinnon. This ensures the earliest possible reinnervation. Reconstruction of the primary lesion is particularly important for the treatment of neuropathic pain.

b) C8/Th1 Lesion

Isolated injuries of the lower roots are generally much less frequent but are also associated with a much worse prognosis. The time of denervation is also decisive here. In addition to the reconstruction of the nerve injury, it is particularly important to prevent the atrophy of the denervated muscles by external treatment for as long as possible. If this is not successful, a contralateral C7 transfer and then a free functional muscle transfer can be performed as a last resort (see Sect. 3.8.1).

Elective amputation and subsequent fitting with a bionic prosthesis is still a controversial approach. It is important to note that a prosthetic fitting at the glenohumeral level, similarly to a complete brachial plexus lesion, shows the worst results. Smaller prostheses, i.e., prostheses positioned further distally, offer significantly better results. Irrespective of this, however, bionic prostheses are also dependent on a nerve supply for control. These often have to be provided by reconstructive surgery.

3.8.1 Functional Free Muscle Transfer

If a plexus lesion fails to regenerate satisfactorily, if there are basically no axon donors available, or if treatment is delayed, the possibility of free functional muscle transfer is available. In a first procedure, a contralateral C7 transfer with long suralis or saphenous nerve grafts is performed. Subsequently, the progression of the Tinel-Hofmann's sign is monitored over several months. Once the axons have reached the

end of the grafts, a free, vascularized muscle transplant is performed. As a rule, the gracilis muscle and the adductor longus muscle are used. Depending on the desired function, up to two muscles can be transplanted in order to perform, usually opposite, functions, such as finger flexion and extension.

3.9 Peripheral Nerve Surgery in Spinal Cord Injuries

Surgical treatment of a central nervous system injury is still limited to acute care. However, depending on the lesion, reconstruction options have recently been found in the peripheral nervous system. Thus, in the case of lesions at the level of C6/C7 or C7/C8, peripheral nerve transfer can be performed to achieve partial reinnervation of the muscles. Although the techniques for this have been known for a long time, the fields of application have only been recognized in recent years.

The most common application is a reconstruction of elbow extension, wrist extension, finger extension, and flexion. Especially elbow extension is of decisive importance for a wheelchair-bound patient. The main advantage of a nerve transfer is the lack of immobilization in a cast. Disadvantages include the long period of time until regeneration. The treatment can be complemented by additional muscle-tendon transfers.

4 Conclusions

The possibilities of peripheral nerve surgery have expanded in recent years and will continue to do so. The so-called paradigm-shift introduced by nerve transfers seems to have moved the spotlight from the question “how to reconstruct a nerve defect” onto the question “how to provide axons to a denervated muscle as fast as possible.” As a surgeon, a step-by-step approach seems to be more and more crucial. The current situation of the patient should be known exactly preoperatively. In many cases, however, it can only be completely clarified intraoperatively. By using the most diverse possibilities, the individual treatment goal must then be achieved. Old and established procedures should not be forgotten due to the rapid progress and the new development of procedures and products to improve function, but at the same time one must remain open for innovations with the idea in mind that even today’s established procedures were critically questioned and scrutinized 50 years ago.

References

- Abdullah S, Ahmad AA, Lalonde D (2020) Wide awake local anesthesia no tourniquet forearm triple tendon transfer in radial nerve palsy. *Plast Reconstr Surgery Glob Open* 8:e3023. <https://doi.org/10.1097/GOX.0000000000003023>
- Al-Qattan MM (2003) Latissimus dorsi transfer for external rotation weakness of the shoulder in obstetric brachial plexus palsy. *J Hand Surg Am* 28 B. [https://doi.org/10.1016/S0266-7681\(02\)00393-5](https://doi.org/10.1016/S0266-7681(02)00393-5)

- Apel PJ, Alton T, Northam C et al (2009) How age impairs the response of the neuromuscular junction to nerve transection and repair: an experimental study in rats. *J Orthop Res* 27. <https://doi.org/10.1002/jor.20773>
- Askar I, Sabuncuoglu BT, Yormuk E, Saray A (2001) The fate of neurotization techniques on reinnervation after denervation of the gastrocnemius muscle: an experimental study. *J Reconstr Microsurg* 17. <https://doi.org/10.1055/s-2001-16027>
- Bak M, Gutlowska O, Wagner E, Gosk J (2017) The role of chitin and chitosan in peripheral nerve reconstruction. *Polym Med* 47. <https://doi.org/10.17219/pim/75653>
- Barbour J, Yee A, Kahn LC, MacKinnon SE (2012) Supercharged end-to-side anterior interosseous to ulnar motor nerve transfer for intrinsic musculature reinnervation. *J Hand Surg Am*:37. <https://doi.org/10.1016/j.jhsa.2012.07.022>
- Bateman JE (1955) *The shoulder and environs*, St Louis, C V Mosby Co 162
- Belcher HJCR, Pandya AN (2000) Centro-central union for the prevention of neuroma formation after finger amputation. *J Hand Surg Am* 25 B. <https://doi.org/10.1054/jhsb.2000.0372>
- Bertelli JA, Ghizoni MF (2011) Very distal sensory nerve transfers in high median nerve lesions. *J Hand Surg Am* 36. <https://doi.org/10.1016/j.jhsa.2010.11.049>
- Bertelli JA, Ghizoni MF (2016) Results of spinal accessory to suprascapular nerve transfer in 110 patients with complete palsy of the brachial plexus. *J Neurosurg Spine* 24:990–995. <https://doi.org/10.3171/2015.8.SPINE15434>
- Bertelli JA, Soldado F, Rodríguez-Baeza A, Ghizoni MF (2018) Transfer of the motor branch of the abductor Digiti Quinti for Thenar muscle Reinnervation in high median nerve injuries. *J Hand Surg Am* 43. <https://doi.org/10.1016/j.jhsa.2017.08.009>
- Bertleff MJOE, Meek MF, Nicolai JPA (2005) A prospective clinical evaluation of biodegradable Neurolac nerve guides for sensory nerve repair in the hand. *J Hand Surg Am* 30. <https://doi.org/10.1016/j.jhsa.2004.12.009>
- Bigland-Ritchie B, Zijdwind I, Thomas CK (2000) Muscle fatigue induced by stimulation with and without doublets. *Muscle Nerve* 23:1348–1355. [https://doi.org/10.1002/1097-4598\(200009\)23:9<1348::aid-mus5>3.0.co;2-0](https://doi.org/10.1002/1097-4598(200009)23:9<1348::aid-mus5>3.0.co;2-0)
- Boldrey E (1943) Amputation neuroma in nerves implanted in bone. *Ann Surg* 118:1052
- Bontioti E, Dahlin LB (2009) Chapter 12 mechanisms underlying the end-to-side nerve regeneration. *Int Rev Neurobiol* 87
- Brooks DN, Weber RV, Chao JD et al (2012) Processed nerve allografts for peripheral nerve reconstruction: a multicenter study of utilization and outcomes in sensory, mixed, and motor nerve reconstructions. *Microsurgery* 32. <https://doi.org/10.1002/micr.20975>
- Brunelli G (1980) Neurolysis and free microvascular omentum transfer in the treatment of post-actinic palsies of the brachial plexus. *Int Surg* 65:515
- Brunelli G, Brunelli LM (1980) Direct neurotization of severely damaged denervated muscles. *Int Surg* 65
- Brunelli G, Brunelli F (1985) Surgical treatment of actinic brachial plexus lesions: free microvascular transfer of the greater omentum. *J Reconstr Microsurg* 1:197–200
- Burke SN, Barnes CA (2006) Neural plasticity in the ageing brain. *Nat Rev Neurosci* 7:30–40. <https://doi.org/10.1038/nrn1809>
- Carlstedt T, Anand P, Hallin R et al (2000) Spinal nerve root repair and reimplantation of avulsed ventral roots into the spinal cord after brachial plexus injury. *J Neurosurg* 93. <https://doi.org/10.3171/spi.2000.93.2.0237>
- Chen L, Gu YD, Hu SN et al (2007) Contralateral C7 transfer for the treatment of brachial plexus root avulsions in children—a report of 12 cases. *J Hand Surg Am* 32. <https://doi.org/10.1016/j.jhsa.2006.05.013>
- Cho MS, Rinker BD, Weber RV et al (2012) Functional outcome following nerve repair in the upper extremity using processed nerve allograft. *J Hand Surg Am* 37. <https://doi.org/10.1016/j.jhsa.2012.08.028>
- Chuang DC, Wei FC, Noordhoff MS (1993) Cross-chest C7 nerve grafting followed by free muscle transplantations for the treatment of total avulsed brachial plexus injuries: a preliminary report. *Plast Reconstr Surg* 92:717

- Clark WL, Trumble TE, Swiontkowski MF, Tencer AF (1992) Nerve tension and blood flow in a rat model of immediate and delayed repairs. *J Hand Surg Am* 17:677–687
- Colonna M, Russo A, Galeano M et al (2015) “Babysitting” procedures in proximal nerve trunk injuries: two case reports and a review. *Plast Aesthetic Res* 2. <https://doi.org/10.4103/2347-9264.160888>
- Cooney WP (1988) Tendon transfer for median nerve palsy. *Hand Clin* 4:155–165
- Craft RO, Duncan SFM, Smith AA (2007) Management of recurrent carpal tunnel syndrome with microneurolysis and the hypothenar fat pad flap. *Hand* 2. <https://doi.org/10.1007/s11552-007-9025-7>
- Craig R, Padrón R (2004) Molecular structure of the sarcomere. *Myology* 3:129–144
- Dahlin LB, Zhao Q, Bjursten LM (1995) Nerve regeneration in silicone tubes: distribution of macrophages and interleukin-1 β in the formed fibrin matrix. *Restor Neurol Neurosci* 8:199–203
- de Mendonça CM, Gepp R, Lima FL, Gushiken A (2020) Intercoastal to musculocutaneous nerve transfer in patients with complete traumatic brachial plexus injuries: case series. *Acta Neurochir* 162:1907–1912. <https://doi.org/10.1007/s00701-020-04433-3>
- Domeshek LF, Novak CB, Patterson JMM et al (2019) Nerve transfers—a paradigm shift in the reconstructive ladder. *Plast Reconstr Surg—Glob Open* 7. <https://doi.org/10.1097/gox.0000000000002290>
- Ehlers MD (2004) Deconstructing the axon: Wallerian degeneration and the ubiquitin–proteasome system. *Trends Neurosci* 27:3–6
- Enoka RM, Fuglevand AJ (2001) Motor unit physiology: some unresolved issues. *Muscle Nerve* 24:4–17. [https://doi.org/10.1002/1097-4598\(200101\)24:1<4::aid-mus13>3.0.co;2-f](https://doi.org/10.1002/1097-4598(200101)24:1<4::aid-mus13>3.0.co;2-f)
- Flores LP, Socolovsky M (2016) Phrenic nerve transfer for reconstruction of elbow extension in severe brachial plexus injuries. *J Reconstr Microsurg* 32:546–550. <https://doi.org/10.1055/s-0036-1583302>
- Goldstein SA, Sturim HS (1985) Intraosseous nerve transposition for treatment of painful neuro-mas. *J Hand Surg Am* 10. [https://doi.org/10.1016/S0363-5023\(85\)80120-9](https://doi.org/10.1016/S0363-5023(85)80120-9)
- Gottschalk HP, Bindra RR (2012) Late reconstruction of ulnar nerve palsy. *Orthop Clin North Am* 43
- Gu YD (1989) Cervical nerve root transfer from the healthy side in the treatment of brachial plexus root avulsion. *Zhonghua Yi Xue Za Zhi* 69
- Gu YD (2007) Contralateral C7 root transfer over the last 20 years in China. *Chin Med J* 120. <https://doi.org/10.1097/00029330-200707010-00001>
- Gu YD, Ma MK (1996) Use of the phrenic nerve for brachial plexus reconstruction. *Clin Orthop Relat Res*:119–121. <https://doi.org/10.1097/00003086-199602000-00016>
- Gu Y, Xu J, Chen L et al (2002) Long-term outcome of contralateral C7 transfer: a report of 32 cases. *Chin Med J* 115
- Hacker V (1908) Erfolgreich operativ behandelte Cucullarislaehmung. *Verein der Aerzte in Steiermark. Wien Klin Wochenschr* 21:1314
- Higgins JP, Fisher S, Serletti JM, Orlando GS (2002) Assessment of nerve graft donor sites used for reconstruction of traumatic digital nerve defects. *J Hand Surg Am* 27:286–292. <https://doi.org/10.1053/jhsu.2002.31154>
- Isaacs J, Ugwu-Oju O (2016) High median nerve injuries. *Hand Clin* 32
- Kakinoki R, Nishijima N, Ueba Y et al (1995) Relationship between axonal regeneration and vascularity in tubulation—an experimental study in rats. *Neurosci Res* 23. [https://doi.org/10.1016/0168-0102\(95\)90009-8](https://doi.org/10.1016/0168-0102(95)90009-8)
- Kehoe S, Zhang XF, Boyd D (2012) FDA approved guidance conduits and wraps for peripheral nerve injury: a review of materials and efficacy. *Injury* 43
- Koriem E, El-Mahy MM, Atiyya AN, Diab RA (2020) Comparison between supercharged ulnar nerve repair by anterior interosseous nerve transfer and isolated ulnar nerve repair in proximal ulnar nerve injuries. *J Hand Surg Am* 45. <https://doi.org/10.1016/j.jhsa.2019.11.005>
- Kovačič U, Žele T, Tomšič M et al (2012) Influence of breaching the connective sheaths of the donor nerve on its myelinated sensory axons and on their sprouting into the end-to-side coapted nerve in the rat. *J Neurotrauma* 29. <https://doi.org/10.1089/neu.2011.2298>

- Küllmer K, Reimers C, Eysel P, Harland U (2008) Sonographische Verlaufskontrolle nach experimenteller Muskeldenerverung. *Ultraschall der Medizin* 17. <https://doi.org/10.1055/s-2007-1003186>
- Lahiji FA, Tahririan MA, Karami M et al (2017) Transfer of pectoralis major to subscapularis in the Management of Brachial Plexus Birth Palsy Sequels. *J Pediatr Orthop* 37:305–310. <https://doi.org/10.1097/BPO.0000000000000648>
- Leblebicioglu G, Ayhan C, Firat T et al (2016) Recovery of upper extremity function following endoscopically assisted contralateral C7 transfer for obstetrical brachial plexus injury. *J Hand Surg Eur* 41. <https://doi.org/10.1177/1753193416638999>
- Leechavengvongs S, Ngamlamiat K, Malungpaishrope K et al (2011) End-to-side radial sensory to median nerve transfer to restore sensation and relieve pain in C5 and C6 nerve root avulsion. *J Hand Surg Am* 36. <https://doi.org/10.1016/j.jhsa.2010.10.009>
- Lin H, Hou C, Chen D (2011) Contralateral C7 transfer for the treatment of upper obstetrical brachial plexus palsy. *Pediatr Surg Int* 27. <https://doi.org/10.1007/s00383-011-2894-4>
- Littler JW (1949) Tendon transfers and arthrodeses in combined median and ulnar nerve paralysis. *JBJS* 31:225–234
- Liu HM (1992) The role of extracellular matrix in peripheral nerve regeneration: a wound chamber study*. *Acta Neuropathol*
- Longo FM, Hayman EG, Davis GE et al (1984) Neurite-promoting factors and extracellular matrix components accumulating in vivo within nerve regeneration chambers. *Brain Res* 309:105–117
- Lundborg G, Rosen B, Dahlin L et al (1997) Tubular versus conventional repair of median and ulnar nerves in the human forearm: early results from a prospective, randomized, clinical study. *J Hand Surg Am* 22. [https://doi.org/10.1016/S0363-5023\(05\)80188-1](https://doi.org/10.1016/S0363-5023(05)80188-1)
- Lundborg G, Rosén B, Dahlin L et al (2004) Tubular repair of the median or ulnar nerve in the human forearm: a 5-year follow-up. *J Hand Surg Am* 29 B. <https://doi.org/10.1016/j.jhsb.2003.09.018>
- Lurje A (1948) Concerning surgical treatment of traumatic injury to the upper division of the brachial plexus (Erb's type). *Ann Surg* 127:317–326
- Ma J, Novikov LN, Kellerth J, Wiberg M (2003) Early nerve repair after injury to the postganglionic plexus: an experimental study of sensory and motor neuronal survival in adult rats. *Scand J Plast Reconstr Surg Hand Surg* 37(1):1–9. <https://doi.org/10.1080/alp.37.1.1.9>
- Mackinnon SE, Novak CB (1999) Nerve transfers: new options for reconstruction following nerve injury. *Hand Clin* 15
- Mackinnon SE, Novak CB, Myckatyn TM, Tung TH (2005) Results of reinnervation of the biceps and brachialis muscles with a double fascicular transfer for elbow flexion. *J Hand Surg Am* 30. <https://doi.org/10.1016/j.jhsa.2005.05.014>
- Martins RS, Siqueira MG, Heise CO et al (2015) Interdigital direct neuroorrhaphy for treatment of painful neuroma due to finger amputation. *Acta Neurochir* 157. <https://doi.org/10.1007/s00701-014-2288-1>
- Mathoulin C, Bahm J, Roukoz S (2000) Pedicled hypothenar fat flap for median nerve coverage in recalcitrant carpal tunnel syndrome. *Hand Surg* 5:33–40
- Mayer L (1927) Transplantation of the trapezius for paralysis of the abductors of the arm. *JBJS* 9: 412–420
- Millesi H (1973) Microsurgery of peripheral nerves. *Hand* 5:157–160
- Millesi H (1985a) Peripheral nerve repair: terminology, questions, and facts. *J Reconstr Microsurg* 2. <https://doi.org/10.1055/s-2007-1007042>
- Millesi H (1985b) Pain syndromes after nerve repair. Treatment by transplantation of gliding tissue. In: the 8th symposium of the international society of reconstructive microsurgery
- Millesi H (1997) Neurolysis. *Brachial Plexus Hand Up Extrem* 14
- Millesi H, Schmidhammer R (2008) Nerve Fiber transfer by end-to-side Coaptation. *Hand Clin* 24
- Millesi H, Ganglberger J, Berger A (1967) Interfasciculäre Nerven transplantation mit Hilfe der mikrochirurgischen Technik. *Transact. In: 4th International Congress of Plastic and Reconstructive Surgery, Rome*

- Millesi H, Berger A, Meissl G (1972a) Experimentelle Untersuchungen zur heilung durchtrennter peripherer nerven. *Chir Plast* 1:174–206
- Millesi H, Meissl G, Berger A (1972b) The interfascicular nerve-grafting of the median and ulnar nerves. *J Bone Joint Surg Am* 54. <https://doi.org/10.2106/00004623-197254040-00004>
- Millesi H, Zöch G, Rath T (1990) The gliding apparatus of peripheral nerve and its clinical significance. *Ann Chir la Main* 9. [https://doi.org/10.1016/S0753-9053\(05\)80485-5](https://doi.org/10.1016/S0753-9053(05)80485-5)
- Millesi H, Rath T, Reihnsner R, Zoch G (1993) Microsurgical neurolysis: its anatomical and physiological basis and its classification. *Microsurgery* 14. <https://doi.org/10.1002/micr.1920140703>
- Millesi W, Schobel G, Bochsansky T (1994) Subpectoral gliding tissue flap. *Plast Reconstr Surg* 93. <https://doi.org/10.1097/00006534-199404000-00029>
- Namazi H, HajiVandi S (2017) Supinator to ulnar nerve transfer via in situ anterior interosseous nerve bridge to restore intrinsic muscle function in combined proximal median and ulnar nerve injury: a novel cadaveric study. *J Surg Res* 211. <https://doi.org/10.1016/j.jss.2016.12.003>
- Narakas AO (1984a) Thoughts on neurotization or nerve transfers in irreparable nerve lesions. *Clin Plast Surg* 11:153–159
- Narakas AO (1984b) Operative treatment for radiation-induced and metastatic brachial plexopathy in 45 cases, 15 having an omentoplasty. *Bull Hosp Jt Dis Orthop Inst* 44:354–375
- Oberlin C, Béal D, Leechavengvongs S et al (1994) Nerve transfer to biceps muscle using a part of ulnar nerve for C5–C6 avulsion of the brachial plexus: anatomical study and report of four cases. *J Hand Surg Am* 19. [https://doi.org/10.1016/0363-5023\(94\)90011-6](https://doi.org/10.1016/0363-5023(94)90011-6)
- Oberlin C, Durand S, Belheyar Z et al (2009) Nerve transfers in brachial plexus palsies. *Chir Main* 28
- Papalia I, Cardaci A, D'Alcontres FS et al (2007) Selection of the donor nerve for end-to-side neurorrhaphy. *J Neurosurg* 107. <https://doi.org/10.3171/JNS-07/08/0378>
- Papalia I, Magaouda L, Righi M et al (2016) Epineurial window is more efficient in attracting axons than simple coaptation in a sutureless (cyanoacrylate-bound) model of end-to-side nerve repair in the rat upper limb: functional and morphometric evidences and review of the literature. *PLoS One* 11. <https://doi.org/10.1371/journal.pone.0148443>
- Park DM, Shon SK, Kim YJ (2000) Direct muscle neurotization in rat soleus muscle. *J Reconstr Microsurg* 16. <https://doi.org/10.1055/s-2000-7349>
- Pillen S, van Alfen N (2011) Skeletal muscle ultrasound. *Neurol Res* 33
- Pondaag W, Gilbert A (2008) Results of end-to-side nerve coaptation in severe obstetric brachial plexus lesions. *Neurosurgery* 62. <https://doi.org/10.1227/01.neu.0000317314.54450.79>
- Poppler LH, Davidge K, Lu JCY et al (2015) Alternatives to sural nerve grafts in the upper extremity. *Hand (N Y)* 10:68–75. <https://doi.org/10.1007/s11552-014-9699-6>
- Ralston E, Ploug T, Kalhovde J, Lomo T (2001) Golgi complex, endoplasmic reticulum exit sites, and microtubules in skeletal muscle fibers are organized by patterned activity. *J Neurosci* 21(3): 875–883. <https://doi.org/10.1523/JNEUROSCI.21-03-00875.2001>
- Ray WZ, Chang J, Hawasli A et al (2015) Motor nerve transfers: a comprehensive review. *Neurosurgery* 78
- Reisman NR, Dellon AL (1983) The abductor digiti minimi muscle flap: a salvage technique for palmar wrist pain. *Plast Reconstr Surg* 72. <https://doi.org/10.1097/00006534-198312000-00025>
- Rose EH, Norris MS, Kowalski TA et al (1991) Palmaris brevis turnover flap as an adjunct to internal neurolysis of the chronically scarred median nerve in recurrent carpal tunnel syndrome. *J Hand Surg Am* 16. [https://doi.org/10.1016/S0363-5023\(10\)80096-6](https://doi.org/10.1016/S0363-5023(10)80096-6)
- Saha AK (1967) Surgery of the paralysed and flail shoulder. *Acta Orthop Scand* 38:3–90
- Sammer DM, Kircher MF, Bishop AT et al (2012) Hemi-contralateral C7 transfer in traumatic brachial plexus injuries: outcomes and complications. *J Bone Jt Surg–Ser A* 94. <https://doi.org/10.2106/JBJS.J.01075>
- Schmidhammer R (2014) Ersatzplastiken und sekundäre Verfahren bei inadäquater neurogener Funktionswiederherstellung. In: *Nervenchirurgie*. Springer, Berlin/Heidelberg, pp 329–361
- Schmidhammer R (2019) The functional impact of nerve grafting and peripheral nerve fiber transfer in high ulnar nerve recovery. In: 24th Sunderland Society Meeting. Jerusalem

- Schmidhammer R, Nógrádi A, Szabó A et al (2009) Synergistic motor nerve fiber transfer between different nerves through the use of end-to-side coaptation. *Exp Neurol* 217. <https://doi.org/10.1016/j.expneurol.2009.03.027>
- Schultz RJ, Aiache A (1972) An operation to restore opposition of the thumb by nerve transfer. *Arch Surg* 105:777–779
- Segal D, Cornwall R, Little KJ (2019) Outcomes of spinal accessory-to-suprascapular nerve transfers for brachial plexus birth injury. *J Hand Surg Am* 44:578–587. <https://doi.org/10.1016/j.jhsa.2019.02.004>
- Siemionow M, Sonmez E (2007) Nerve allograft transplantation: a review. *J Reconstr Microsurg* 23
- Stamatoukou A, Papadogeorgou E, Zhang Z et al (2006) Phrenic nerve neurotization of the musculocutaneous nerve with end-to-side neurorrhaphy: a short report in a rabbit model. *Microsurgery* 26. <https://doi.org/10.1002/micr.20238>
- Stefancic M, Vidmar G, Blagus R (2016) Long-term recovery of muscle strength after denervation in the fibular division of the sciatic nerve. *Muscle Nerve* 54. <https://doi.org/10.1002/mus.25103>
- Steindler A (1919) Operative treatment of paralytic conditions of the upper extremity. *JBJS* 1:608–619
- Stoll G, Müller HW (1999) Nerve injury, axonal degeneration and neural regeneration: basic insights. *Brain Pathol* 9:313–325
- Stoll G, Jander S, Myers RR (2002) Degeneration and regeneration of the peripheral nervous system: from Augustus Waller's observations to neuroinflammation. *J Peripher Nerv Syst* 7:13–27
- Strickland JW, Idler RS, Lourie GM, Plancher KD (1996) The hypothenar fat pad flap for management of recalcitrant carpal tunnel syndrome. *J Hand Surg Am* 21. [https://doi.org/10.1016/S0363-5023\(96\)80201-2](https://doi.org/10.1016/S0363-5023(96)80201-2)
- Terzis JK, Karypidis D (2009) Outcomes of direct muscle neurotization in pediatric patients with facial paralysis. *Plast Reconstr Surg* 124
- Terzis JK, Kostopoulos E (2010) Our experience with secondary reconstruction of external rotation in obstetrical brachial plexus palsy. *Plast Reconstr Surg* 126. <https://doi.org/10.1097/PRS.0b013e3181e603d3>
- Tham SKY, Ireland DCR, Riccio M, Morrison WA (1996) Reverse radial artery fascial flap: a treatment for the chronically scarred median nerve in recurrent carpal tunnel syndrome. *J Hand Surg Am* 21. [https://doi.org/10.1016/S0363-5023\(96\)80202-4](https://doi.org/10.1016/S0363-5023(96)80202-4)
- Thomas CK, Johansson RS, Bigland-Ritchie B (1999) Pattern of pulses that maximize force output from single human thenar motor units. *J Neurophysiol* 82:3188–3195
- Tinel J (1916) *Les blessures des nerfs: sémiologie des lésions nerveuses périphériques par blessures de guerre*. Masson et cie
- Tos P, Battiston B, Nicolino S et al (2007) Comparison of fresh and predegenerated muscle-vein-combined guides for the repair of rat median nerve. *Microsurgery* 27. <https://doi.org/10.1002/micr.20306>
- Tos P, Battiston B, Ciclamini D et al (2012) Primary repair of crush nerve injuries by means of biological tubulization with muscle-vein-combined grafts. *Microsurgery*:32. <https://doi.org/10.1002/micr.21957>
- Uhlschmid G (1978) Eine neue Anwendung des frei transplantierten Omentums
- Vernadakis AJ, Humphreys DB, Mackinnon SE (2004) Distal anterior interosseous nerve in the recurrent motor branch graft for reconstruction of a median nerve neuroma-in-continuity. *J Reconstr Microsurg* 21:7–11
- Waller AV (1850) XX. Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibres. *Philos Trans R Soc London*:423–429
- Wang Y, Zhu S (1997) Transfer of a branch of the anterior interosseous nerve to the motor branch of the median nerve and ulnar nerve. *Chin Med J* 110
- Wang M, Xu W, Zheng M et al (2011) Phrenic nerve end-to-side neurotization in treating brachial plexus avulsion: an experimental study in rats. *Ann Plast Surg* 66. <https://doi.org/10.1097/SAP.0b013e3181f322fd>

- Wang H, Zhao Q, Zhao W et al (2012) Repairing rat sciatic nerve injury by a nerve-growth-factor-loaded, chitosan-based nerve conduit. *Biotechnol Appl Biochem* 59. <https://doi.org/10.1002/bab.1031>
- Williams LR, Longo FM, Powell HC et al (1983) Spatial-temporal progress of peripheral nerve regeneration within a silicone chamber: parameters for a bioassay. *J Comp Neurol* 218:460–470
- Windisch A, Gundersen K, Szabolcs MJ, Gruber H, Lømo T (1998) Fast to slow transformation of denervated and electrically stimulated rat muscle. *J Physiol* 510(Pt 2):623–632. <https://doi.org/10.1111/j.1469-7793.1998.623bk.x>
- Yamamoto R, Motomiya M, Sakurai K et al (2015) Application of free temporoparietal fascial flap for recurrent neural adhesion of superficial radial nerve—a case report. *Microsurgery* 35. <https://doi.org/10.1002/micr.22446>
- Yang W, Yang J, Yu C, Gu Y (2014) End-to-side neurotization with different donor nerves for treating brachial plexus injury: an experimental study in a rat model. *Muscle Nerve* 50. <https://doi.org/10.1002/mus.24110>
- Yin K, Divakar P, Hong J, et al (2018) Freeze-cast Porous Chitosan Conduit for Peripheral Nerve Repair. In: *MRS Advances*
- Zhao Q, Dahlin LB, Kanje M, Lundborg G (1992) Specificity of muscle reinnervation following repair of the transected sciatic nerve: a comparative study of different repair techniques in the rat. *J Hand Surg Am* 17:257–261



Clinical Outcome Measures Following Peripheral Nerve Repair

The Future for Assessment of the Processes and Experiences of Nerve Injury

Matthew Wilcox, Hazel Brown, and Tom Quick

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Abstract

The hope of translational medicine is to identify treatments that improve the rate and quality of neuronal recovery following peripheral nerve injuries (PNI). A major obstacle to the trial of new treatments for PNI is the absence of sensitive and responsive measures of clinical function, which are the ultimate outcome of the biological events of nerve regeneration.

The development of objective clinical measures of nerve regeneration that are sensitive, responsive, and valid is challenging for a number of reasons. The Peripheral Nervous System (PNS) allows us to have complex interactions with our surroundings (for example, the perception of pain, touch, and temperature). Developing objective measures that reflect the multimodal function of the PNS and separate it from psychological influences on that experience is difficult. Secondly, the recovery of function following PNI is dependent upon a number of biological processes at multiple levels including the end organ, the damaged nerve segments, as well as the Central Nervous System (CNS). Clinical measures that quantify these changes and relate them to the recovery of function are not well documented.

The biological process of neural regeneration is often assessed in the research arena through postmortem tissue assessment. However, the harvesting of vital nerve tissue for assessment of neural regeneration often risks the function that has been recovered. Therefore, this is not feasible when studying the biological process of regeneration in humans. This has necessitated the development of noninvasive methods to attain the same degree of assessment of biological regeneration.

In order to assess the biological mechanisms of tissue and cellular regeneration, the relationship of these processes with human clinical experiences needs to

be explained. Correlating these new areas of human assessment will help establish the influence of the complex biological processes that underpin nerve regeneration on the lived experience of nerve injury.

This chapter provides an overview of the clinical, neurophysiological, and imaging assessments that can provide pre-, intra-, and postoperative measures of nerve regeneration. This will inform the development of outcome measures that have the capacity to detect a meaningful clinical response in clinical trials which aim to determine the efficacy of new therapies for PNI.

1 Introduction

The Peripheral Nervous System (PNS) allows us to have complex interactions and experiences with our surrounding environment. Healthy nerve function is important for the control of movement and sensation (pain, touch, and temperature). The autonomic nervous system also plays a critical role. The sympathetic system is responsible for mediating the fight or flight response and background functions such as sweating and nail growth. These actions complement that of the parasympathetic system which control a number of bodily processes such as sexual arousal, salivation, lacrimation, urination, digestion, and defecation. Injury to this complex system is common following blunt or penetrating trauma resulting in a complex mix of functional deficits (Saadat et al. 2011; Brattain 2012). Moreover, the intricate anatomy and diverse range of trauma mechanisms make peripheral nerve injuries (PNI) a heterogeneous pathology to study. Successful nerve regeneration is only possible through a number of biological processes that occur at multiple levels; the Central Nervous System (CNS), injured nerve segments, glial cells, extracellular matrix molecules, and end organs are all involved. The hope of translational medicine is to identify treatments that modulate one or more of these processes to improve the rate and quality of recovery following PNI. A number of treatments for PNI have been developed in preclinical models but the limitations of current outcome measures has hindered clinical translation (Boyd and Gordon 2003; Gordon et al. 2009; Li et al. 2014; Willand et al. 2015; Sullivan et al. 2016). In addition, it remains unclear to what extent the cellular and molecular mechanisms that underpin nerve regeneration in rodents are mimicked in humans. Addressing these issues will be key to effective translation of new therapies to improve peripheral nerve repair.

Clinical, neurophysiological, and imaging assessments are useful in characterizing nerve injuries and determining their severity (Koltzenburg and Bendszus 2004; Campbell 2008; Smith and Knight 2011; Simon et al. 2016; Kakkar et al. 2018). However, outcome measures that are sensitive and responsive to the biological processes associated with nerve regeneration in isolation from other aspect of human clinical medicine such as psychology are not well documented. Moreover, many assessments fail to account for the multifaceted function of the PNS (Rayner et al. 2019; Wilcox et al. 2020). Evaluations of the degree of biologic recovery of PNI are pertinent for a number of reasons. Firstly, this will allow subclinical degrees

of change to be detected; long before clinical manifestations of muscle reinnervation are evident. This will provide invaluable information to identify cases where surgical intervention may offer clinical benefit. Secondly, it will allow the biological efficacy of new treatments for PNI to be established in isolation from other aspects of human clinical medicine such as psychology (Ultee et al. 2013). Understanding the links between these measures with objective and subjective measurements of muscular function will help drive clinical translation of therapies to improve peripheral nerve repair (discussed in more detail in chapter ► [“Rehabilitation of Nerve Injuries”](#)).

This chapter provides a comprehensive overview of how damage to components of the PNS and the biological response that ensues may facilitate nerve regeneration and functional recovery. The second part aims to relate these biological changes to clinical outcome measures of nerve regeneration and highlight opportunities to develop novel sensitive outcome evaluations that can detect a meaningful clinical response in clinical trials of therapies that hope to enhance human peripheral nerve repair.

2 Peripheral Nerve Anatomy

The PNS consists of neuronal cells, glial cells, and stromal cells. Peripheral nerves relay signals from the spinal cord to the rest of the body and from sensory receptors to the CNS. Nerve trunks principally comprise of efferent and afferent neurons. Efferent fibers can be made up of motor and autonomic fibers which receive signals from dendrites at the spinal cord primarily through the neurotransmitters acetylcholine, glutamate (e.g., from sensory neurons), glycine (inhibitory neurons) and several others. Afferent neurons can be somatic or autonomic in origin and receive their input from the dendrite of specialized cells such as Pacinian corpuscles located in the skin which detect fine sensation.

Schwann cells, the glial cells of the peripheral nervous system, surround individual axons and have a number of important functions. These cells produce myelin which allows saltatory conduction along myelinated fibers. A α motor fibers represent the most heavily myelinated followed by A β afferent muscle spindle fibers. Both fibers have a conduction velocity of around 30–120 m/s. On the contrary, C fibers are unmyelinated and therefore the slowest conducting with 1–2 m/s. These fibers relay information from the periphery to the CNS about pain, temperature and also make up the post-ganglionic sympathetic system. The properties of the different fibers in the PNS are summarized in Table 1. Beyond myelin production, Schwann cells have a close, interdependent relationship with axons (addressed in chapter ► [“Schwann Cells in Nerve Repair and Regeneration”](#)).

Non-neuronal cells and connective tissue surrounding the nerve trunk have an important function in providing a structural scaffold for the nerve and adapting it for its function. An endoneurium surrounds individual axons. Groups of axons are surrounded by a layer of loose connective tissue called the perineurium which is composed mainly of collagen and elastin. Surrounding this is a layer of the epineurium which contains a network of cellular, lymphatic and vascular structures that are

Table 1 The properties of different nerve fiber types (Simon et al. 2016)

Fiber class	Myelin sheath	Diameter (mm)	Conduction velocity (m/s)	Spinal cord tract	Location	Function
A α	Yes	6-22	30-120	Ipsilateral dorsal column	Efferent to muscles	Motor
A β	Yes	6-22	30-120	Contralateral spinothalamic tract	Afferent from skin and joints	Tactile, proprioception
A γ	Yes	3-8	15-35	Ipsilateral dorsal column	Efferent to muscle spindles	Muscle tone
A δ	Yes	1-4	5-30	Contralateral spinothalamic tract	Afferent sensory nerves	Pain, cold, temperature, touch
B	Yes	1-3	3-15	Preganglionic	Preganglionic sympathetic	A number of autonomic functions
sC	No	0.3-1.3	0.7-1.3	–	Postganglionic sympathetic	A number of autonomic functions
dC	No	0.4-1.2	0.1-2.0	Contralateral spinothalamic tract	Afferent sensory nerves	A number of autonomic functions Pain, warm, temperature, touch

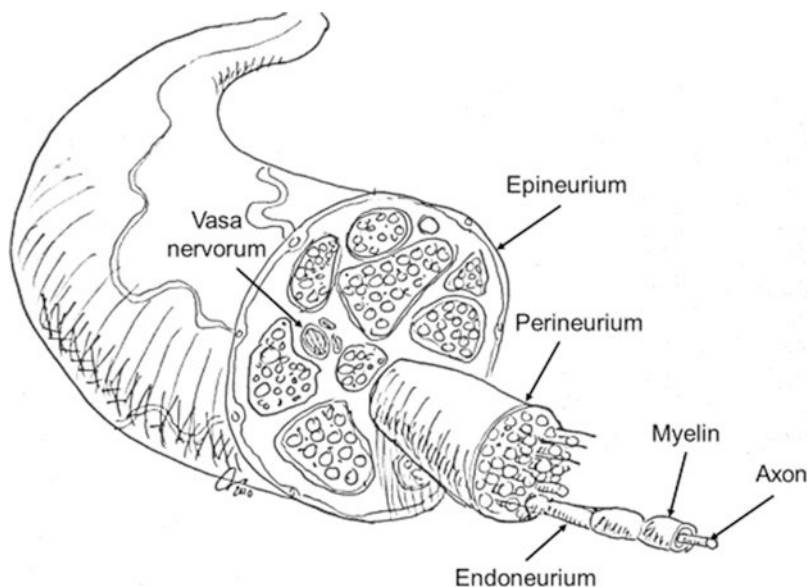


Fig. 1 Anatomy of the nerve trunk

important for the normal functioning of nerves and their response to injury. A visual representation of the nerve trunk and its corresponding microanatomy is shown in Fig. 1. The extent of damage to this network is important in classifying nerve injuries (Geuna et al. 2009).

3 Classifications of Peripheral Nerve Injuries

Nerve tissue is viscoelastic. Therefore, in closed injuries the severity (grade) of nerve injury largely depends upon the magnitude, rate of application, and duration of the forces applied to the nerve trunk. A number of systems exist for the classification of PNI which are based upon the extent of damage to the microanatomy of the nerve trunk. The Seddon and Sunderland classification systems have been widely deployed to assist in determining the severity, prognosis, and treatment strategy of PNI (Table 2) (Seddon 1943; Sunderland 1951; Mackinnon and Dellon 1988). Mackinnon and Dellon modified Sunderland's classification to include a mixed injury type to better reflect clinical practice (Mackinnon and Dellon 1988). Millesi's classification goes one step further to relating the severity of focal nerve injuries requiring reconstruction to the surgical steps necessary for reconstruction (Millesi 1985).

Grade I injuries (neurapraxia) may be secondary to a single or persistent event. This type of injury is particularly prevalent in anatomical locations where nerves may be susceptible to stretch or compression such as in osseofascial tunnels

Table 2 A summary of the Seddon and Sunderland classification systems and their likely functional outcomes and required surgical intervention (Mackinnon and Dellon 1988; Mydlarz and Boahene 2013; Kaya and Sarikcioglu 2015)

Seddon classification	Neuropathology	Sunderland classification	Surgical intervention required?	Likely functional outcome
Neurapraxia	Segmental demyelination	Grade I	Not unless the pathology is persistent	Complete recovery most likely without surgical intervention
Axonotmesis	Transection of the axons but the remaining endoneurium, perineurium and epineurium intact	Grade II	Not likely	Often complete recovery without surgical intervention
Axonotmesis	Transection of axons, endoneurium with preservation of the perineurium and epineurium	Grade III	Maybe	Unlikely complete without surgical intervention
Axonotmesis	Transection of axons, endoneurium, perineurium with preservation of the epineurium	Grade IV	Yes	Poor without surgical intervention
Neurotmesis	Disruption of the entire nerve trunk	Grade V	Yes	Requires surgical intervention
Mixed	Combination of Sunderland Grade I to Grade V	Grade VI	Yes	Requires surgical intervention

and following blunt trauma or stretch injuries. Spontaneous recovery almost always ensues in weeks to months following injury. Neurapraxia can be described as a conduction block meaning the nerve is anatomically intact but physiologically broken.

Grade II injuries (axonotmesis) lie within the mild end of the degenerative spectrum. Here, there is discontinuation of the axons but preservation of the surrounding connective tissue usually following blunt trauma. Spontaneous recovery is common and surgical intervention is rarely indicated in the absence of intraneural scar tissue.

In Grade III–Grade V injuries (Grade III and Grade IV: axonotmesis, Grade V: Neurotmesis), there is more widespread disruption to the microanatomy of the nerve trunk. These grades of injuries are more common in blunt, stretch, and/or penetrating trauma. The restoration of function is often not possible without surgical intervention.

While these classification systems may be a useful tool in determining the anatomical extent of nerve damage and likely functional outcomes, they are unlikely to reflect the clinical picture. Injuries often do not damage the nerve trunk uniformly

meaning that these classification systems most likely do not reflect the heterogeneous pathology. It is more likely that nerves demonstrate a mixed classification of nerve injuries as highlighted by the Mackinnon and Dellon's modification to Sunderland's classification (Mackinnon and Dellon 1988; Timothy 2014) (Table 2).

The biological processes that underpin functional recovery depend largely upon the grade of the injury.

4 Neurobiology of Peripheral Nerve Regeneration

An understanding of the biological processes that underpin regeneration and functional recovery in those who have suffered neural trauma is necessary in order to understand and interpret pathological changes on clinical tests.

4.1 Neurapraxia (Grade I Injury)

Pathologically, neurapraxia has a number of potential causes of physiologic dysfunction. The most well characterized is focal demyelination. Degeneration of the myelin sheath impairs conduction of action potentials by changing the mechanical and physical properties of the internodal and paranodal membranes. The capacitance of the membranes increases accompanied by a reduction in the resistance between the paranodal and internodal regions (Bostock and Sears 1976). The result is a deficiency of current which is able to depolarize the next node of Ranvier. The demyelination of the paranodal segment with the increased exposure of the K^+ channels leads to persistent hyperpolarization (Smith and Knight 2011). The activity of the Na^+/K^+ ATPase pump further pushes the resting potential towards the K^+ equilibrium potential (Bostock et al. 1981). The result is the block of conduction leading to clinical signs that are comparable to a lesion that has led to axonal loss.

4.2 Biological Processes that Underpin Neural Regeneration (Grade II–V Injuries)

Neural regeneration is a key biological process in patients recovering from lesions which have resulted in axonal degeneration. A number of cellular and molecular mechanisms at multiple levels are involved: the CNS, cell body along with the proximal and distal stumps.

4.3 Changes at the Central Nervous System

The CNS undergoes rapid and long-lasting remodeling following PNI (Churchill et al. 2001; Hansson and Brismar 2003; Duff 2005). The mechanisms which

underpin plasticity and reorganization in the spinal cord and brain following axon transection are highly complex and poorly understood (Churchill et al. 2001; Hansson and Brismar 2003; Duff 2005). Changes within these systems may result in beneficial adaptive functional changes or may cause defective changes which result in symptoms such as neuropathic pain, hyper-reflexia, and dystonia (Churchill et al. 2001; Hansson and Brismar 2003; Duff 2005). There are currently no effective treatments which can ensure full sensorimotor recovery.

4.4 Changes at the Cell Body and Proximal Stump

Cellular and molecular changes at the cell body and the proximal stump are dependent upon a number of factors such as the severity of the injury and the proximity of the lesion to the cell body. At the site of the nerve injury, there is degeneration back to the previous node of Ranvier which is sometimes referred to as “traumatic degeneration” (Dellon and Mackinnon 1988). This physiological process is identical to Wallerian degeneration which occurs at the distal stump. In severe injuries, degeneration may extend more proximally and include the cell body in which case the whole proximal segment disintegrates and is phagocytosed (Lee and Wolfe 2000; Severinsen and Jakobsen 2009). The nerve cell body reacts to injury through a process known as chromatolysis which involves migration of the nucleus to the cell periphery and Nissl granules from the rough endoplasmic reticulum disintegrating and dispersing (Lee and Wolfe 2000; Severinsen and Jakobsen 2009). Simultaneously, there is proliferation of peri-neuronal glial cells (Lee and Wolfe 2000; Severinsen and Jakobsen 2009). The glial cell processes extend and interrupt synaptic connections to isolate the nerve as it enters a regenerative phase (Lee and Wolfe 2000; Severinsen and Jakobsen 2009).

These events are accompanied by a change in the phenotype of axons and Schwann cells from one that supports myelination and the propagation of action potentials, to one that promotes axonal regeneration and sprouting from the proximal stump (Jessen and Arthur-Farraj 2019; Jessen and Mirsky 2019). The cellular and molecular mechanisms that underpin this reprogramming event have been outlined in chapters ► [“The Immune Response and Implications for Nerve Repair”](#) and ► [“Schwann Cells in Nerve Repair and Regeneration.”](#) Axons sprouting from the proximal stump are reduced in diameter especially when functional connections to appropriate end organs are not reestablished (Giannini et al. 1989; Ikeda and Oka 2012). As axonal regeneration and reinnervation of the end organ ensues, axonal diameter increases although this rarely returns to pre-injury levels (Giannini et al. 1989; Ikeda and Oka 2012). The recovery of the axon and the cell body appear to have some symbiosis; the cell body does not fully recover until functional connections are reestablished with the end organ whilst the final diameter of the regenerating axons depends to a large extent on the recovery of the cell body (Burnett and Zager 2004; Mar et al. 2014).

4.5 Changes at the Distal Stump

Almost immediately after PNI, there is Wallerian degeneration along a proximo-distal gradient (Waller Augustus and Owen 1851; Lubinska 1982). This biological process can be stratified into two distinct stages; a latent phase followed by an aggressive granular phase of neural degeneration (Burnett and Zager 2004). The time course for complete degeneration of the axons takes around 2 weeks (Chaudhry and Cornblath 1992; Gaudet et al. 2011; Rotshenker 2011; Smith and Knight 2011). This process also involves a rapid reprogramming of Schwann cells which shift their phenotype from one that supports myelination to a repair phenotype that is supportive of axonal regeneration (Jessen and Arthur-Farraj 2019; Jessen and Mirsky 2019). The cellular and molecular mechanisms that underpin this shift have been well documented in chapters ► [“Schwann Cells in Nerve Repair and Regeneration”](#) and ► [“The Immune Response and Implications for Nerve Repair”](#) and facilitate a critical step towards creating a microenvironment that is supportive of axonal regeneration. Animal paradigms of chronic denervation have shown that this response is not maintained but decays over time. The resultant incremental delay in reinnervation creates an environment increasingly antagonistic to reinnervation. This has been largely attributed to Schwann cell atrophy and phenotypic changes over time (Jessen and Arthur-Farraj 2019; Jessen and Mirsky 2019).

4.6 Changes at the Neuromuscular Junction and Within the Muscle

Following neural injury, the neuromuscular junction (NMJ) and the target muscle endure significant changes. The NMJ undergoes extensive remodeling with increases in gutter depth, fragmentation, and plate area (Ma et al. 2005). Simultaneously, the muscle begins to atrophy and the diameter of muscle fibers begins to decline with a reduction in muscle fiber conduction velocity (Kraft 1990). There are also a number of changes at cellular and molecular levels; mRNAs coding for nicotinic acetylcholine receptor synthesis and myogenic regulatory factors are upregulated initially followed by a regression back to normal (or subnormal levels) as denervation time increases or reinnervation ensues (Kraft 1990; Voytik et al. 1993; Adams et al. 1995; Weis et al. 2000; Ma et al. 2005, 2007).

The reinnervation of the target muscle has been shown to induce changes in the Major Histocompatibility Complex (MHC) composition at the mRNA and protein level (Mendler et al. 2007; Wu et al. 2014). After denervation, the overall quantity of muscle fibers has been shown to remain constant for up to 1 year in animal models. However, the individual fibers atrophy and change types altering the distribution and proportion of muscle fiber types (Ashley et al. 2007). The direction and extent of this shift is complex and variable within different muscles (Pette and Vrbova 1985; Windisch et al. 1998; Kostrominova et al. 2005). If reinnervation is achieved, the signal generated by the motor cortex is thought to determine the proportion and distribution of muscle fiber types (Hughes et al. 1993).

For many years, it was thought that changes within the muscle were largely responsible for poor functional outcomes in patients where nerve repair was delayed (Fu and Gordon 1995). However, animal models have shown that axons still have the capacity to grow into chronically denervated muscle (Fu and Gordon 1995; Saito and Dahlin 2008). This suggests that in the absence of intraneural scar tissue, changes within the chronically denervated stump are majority contributors to making the environment increasingly antagonistic to nerve regeneration (Ronchi and Raimondo 2017).

In summary, considering clinical observations in the light of findings in animal paradigms of reinnervation, it is thought that optimum functional recovery is largely dependent on a sufficient quantity and quality of axons reinnervating the muscle within 1 year following injury (Dellon and Mackinnon 1988; Mackinnon et al. 2005; Tung and Mackinnon 2010). Beyond this time frame, it is thought that phenotypic changes of Schwann cells, axons, and irreversible degenerative changes at the motor endplate create an environment that becomes increasingly antagonistic to regeneration over time (Tung and Mackinnon 2010; He et al. 2014; Jessen and Mirsky 2019). Despite significant advancements in surgical interventions for the treatment of PNI, muscle reinnervation is often seen by the patient as being functionally incomplete (Scheib and Hoke 2013). A number of treatments have been developed in preclinical models that modulate these biological processes to promote axonal regeneration, preserve denervated muscle, and/or modulate complex remodeling of the CNS (Lee and Wolfe 2000; Faroni et al. 2015). Clinical translation is hindered by the lack of outcome measures that demonstrate sufficient responsiveness and sensitivity to detect and measure these biological changes associated with muscle denervation and reinnervation. The success or failure of these biological events can be monitored to an extent through clinical evaluations of nerve regeneration.

5 Clinical Measurements of Nerve Injury and Regeneration

Clinical assessments are ultimately an evaluation of function and do not measure nerve regeneration in isolation. The Tinel sign, described over a century ago (1917), measures the advancement of regenerating axons by percussing on the skin along the anatomical course of the nerve (Macdonald 1918). It is the only test that can monitor the recovery of axons following a degenerative lesion prior to any end-organ reinnervation.

It should be recognized that there is unlikely to be a linear relationship between the quantity and quality of neuronal regeneration and functional recovery. This is because there are many layers of complexity within the regenerating system. For example, within a certain range (down to around 20% of the original number of axons) a denervated muscle can still become fully reinnervated and may still develop pre-injury peak force (Sobotka and Mu 2013; Hundepool et al. 2018). However, it may be reinnervated by axons which were originally from a differing muscle (a process called axonal confusion) (Pavlov et al. 2008; Seitz et al. 2011). This may lead it to function incorrectly or not be under the patient's easy conscious

control. As a result, the muscle is likely to have reduced gradeability of force, increased fatigue, and co-contract with antagonists. It may also demonstrate decreased proprioception, altered tendon reflex, and overall tension. Moreover, the joints the nerve serves may become stiff and the overlying skin may become dry and painful (Seitz et al. 2011). These peripheral mechanisms are accompanied by parallel central processes demonstrating that there is a highly complex system at study here. The relationship between axonal recovery and subjective measurements of function is also further complicated by psychological and environmental aspects. Normal nerve function in humans allows interaction with our environment such as pain, touch, and temperature. Therefore, it is not surprising that injury to this complex system also result in changes to the way in which one perceives themselves. Clinical assessments should be holistic to mirror the multimodal functions of the PNS. Measures reported by the clinician such as pain, sensibility, motor function, electro-diagnostics, and imaging should be considered alongside patient reported measures (Brown et al. 2018). This is discussed in more detail in chapter ► [“Rehabilitation of Nerve Injuries.”](#) Some of the commonly deployed and recommended clinical measures of functional recovery are discussed in the following sections.

5.1 Sensibility

Sensibility can be defined as the ability to perceive and assimilate sensation. Detection, discrimination, quantification, and identification represent the four levels of sensibility which include all the characteristics of touch, stereognosis, and proprioception. This provides some indication as to the integrity of afferent/sensory neurons which receive their input through dendrites from specialized cells such as Pacinian corpuscles among others. This information is then relayed to the somato-sensory cortex.

The ability of patients to detect static touch and cutaneous pressure is most commonly determined using the Semmes-Weinstein monofilament test and/or the Weinstein Enhanced Sensory Test (WEST) (Weinstein 1993; Jerosch-Herold 2005). These tests have yielded good sensitivity and reliability in detecting changes over time (Jerosch-Herold 2005). The next level of sensibility is discrimination. The most commonly deployed by clinicians is the two-point discriminatory test which is included in the Medical Research Council (MRC) assessment of sensory recovery providing a measurement of recovery across an 8-point scale. However, a number of studies have raised concerns over the sensitivity of this measure (Chassard et al. 1993; Jerosch-Herold 2005).

The final component of sensibility is stereognosis which is defined as the ability to recognize an object without any visual cues. The Shape Texture Identification (STI) test is commonly used and has demonstrated good reliability and sensitivity in monitoring outcomes during the recovery of median and ulnar nerves (Rosen and Lundborg 2003; Jerosch-Herold 2005). Further appraisal of these systems can be found in chapter ► [“Rehabilitation of Nerve Injuries.”](#)

A complex relationship exists between the communication of afferent neurons with the CNS. By extension, this affords numerous challenges when trying to determine what relationship each of these assessments have to the biological processes of nerve regeneration.

5.2 Pain

Nociceptive and neuropathic pain are highly prevalent following PNI. The incidence of neuropathic pain following traumatic injuries that involve the brachial plexus has been reported to be as high as 50% (Ciaramitaro et al. 2010). The symptom of pain is measured in clinical practice by using visual or verbal scale of intensity from 0 to 10 (Delgado et al. 2018). Other scales exist with written descriptors ranging from “no pain” to “worst pain imaginable” (Delgado et al. 2018). The PNI score is a simplified version of this assessment (Birch 2011). However, assessment of intensity in isolation is unlikely to reflect the complexity of the lived experience of pain. The International Association for the Study of Pain (IASP) definition of pain is “an unpleasant sensory and emotional experience associated with actual or potential tissue damage” (1986). This definition reflects that pain goes beyond *severity* alone to better reflect the lived experience of the patients. Outcome measures such as the McGill pain questionnaire go beyond measurements of pain severity to incorporate sensory descriptors and assess temporal changes in pain. Therefore, these questionnaires may better reflect the lived experience of pain. This is discussed in more detail in chapter ► [“Rehabilitation of Nerve Injuries.”](#)

In summary, this symptom is likely to have biological effects at multiple levels as well as psychological impacts which can complicate any attempt to establish outcome measures that relate the biological processes of nerve regeneration with patient function and experience.

5.3 Motor Function

The function of an innervated muscle provides active range of movement (AROM) across a joint. The function of multiple muscles across joints leads to the coordination of movement. The movement across numerous joints allows us to carry out functional tasks. It follows that any assessment of function should reflect each of these components. Measurements of AROM give some indication as to the recovery from paralysis following PNI. The recovery of force often involves measurements of peak volitional force (PVF) as assessed by the MRC grading system of muscle power (Table 3) from 0 to 5. The MRC grading system is universally incorporated into manual muscle testing (MMT) which has shown good reliability in distinguishing between nerve injured and uninjured muscles (Aberg et al. 2007). However, the MRC grading system has a number of limitations. There is some subjectivity in the grading of “normal power.” This ambiguity can make distinguishing between MRC grade 4 and 5 challenging (Table 3). For this reason,

Table 3 The Medical Research Council (MRC) grading system of muscle power

Medical Research Council (MRC) score	Muscle response
0	No movement
1	Flicker of movement
2	Movement with gravity eliminated
3	Movement against gravity only (no resistance)
4	Movement against moderate resistance
5	Normal power

it has been argued that MRC grading of muscle power is an insensitive outcome measure with over 96% of recordings falling within MRC grade 4 (MacAvoy and Green 2007). This has led to the more widespread use of continuous measurements of PVF using handheld dynamometry (Quick et al. 2016).

More recently, qualitative studies have refocused attention onto parameters important to patients. Earlier onset of muscular fatigue, increased co-contractibility, and pain have been identified as pertinent characteristics of reinnervated muscle by patients (Chammas et al. 1997; Brown et al. 2018). This suggests that assessments of recovered motor function should include objective and subjective measurements beyond that of PVF. These findings highlight that it is important to bear in mind how the entire limb is integrated into daily functional activities. The Sollerman Hand function test and Action Research Arm Test (ARAT) are examples of assessments that measure integrated function in hemiplegic patients (Sollerman and Ejekkar 1995; Hsueh et al. 2002; McDonnell 2008; Weng et al. 2010), but they are yet to be validated in a clinical setting of PNI. Patient Reported Outcomes (PROs) are also becoming increasingly utilized globally; resilience and coping strategies are thought to be key predictors of clinical outcomes (Rainey et al. 2014; Jayakumar et al. 2018). A number of strategies for measuring PROs are discussed in ► [“Rehabilitation of Nerve Injuries.”](#)

In summary, many clinical measures of nerve regeneration are subjective and do not fully represent the lived patient experience of muscle reinnervation (Brown et al. 2018). By extension, many of these outcome assessments are insensitive and unresponsive to the biological process of nerve regeneration. Improved objective and subjective metrics that better reflect the lived experience of muscular recovery are needed in order for appropriate outcome measures to be able to detect a meaningful clinical response.

6 Neurophysiological Investigation of Peripheral Nerve Injuries

Neurophysiological changes are evident long before the onset of clinical signs of muscle reinnervation. Nerve Conduction Studies (NCS) and electromyography (EMG) are indispensable tools in the evaluation of PNI. The information provided

by these tests can provide invaluable information to the clinician about the anatomical site of the nerve lesion and its severity. Moreover, changes on these tests correlate with biological processes of nerve regeneration at multiple levels: the denervated muscle, injured nerve segments, and the CNS.

6.1 Nerve Conduction Studies

Nerves and muscles are excitable tissues, therefore their potentials can be induced and recorded. NCS measure changes in the excitability of nerves and muscles following injury. NCS are often the first line of investigation in the case of suspected PNI requiring minimal patient cooperation. Pertinent parameters that are measured during NCS are:

Compound Muscle Action Potential (CMAP): The summated action potentials of all stimulated motor endplates. It is obtained by supra-maximal stimulation of the trunk of the nerve and recording the response of the muscle. The amplitude of the CMAP can be measured as peak to peak or base to peak and is largely determined by the number of functional motor units (MUs) within a muscle, temporal dispersion, magnitude, and duration of single muscle fiber action potentials. The distal latency can be defined as the time between stimulation of the nerve and the onset of the evoked response. The motor conduction velocity can be assessed by the conduction distance divided by the conduction time (Smith and Knight 2011).

Sensory Nerve Action Potential (SNAP): This represents the response of a sensory nerve following electrical stimulation. Recording is often performed using surface electrodes placed over the skin. The use of needle electrodes may be warranted when examining nerves that are difficult to access due to anatomical reasons (e.g., if the nerve is particularly deep to the skin surface). The magnitude of the evoked response is much smaller than the CMAP. Latency of the SNAP can be determined by the time taken for the initial deflection from baseline following stimulation. The sensory nerve conduction velocity can be calculated as the conduction distance from the stimulating electrode divided by the latency (Lowitzsch et al. 1977).

6.2 Electromyography

The response of the muscle to electrical impulses travelling along the nerves can be characterized using EMG. This involves recording the electrical activity of the affected muscle often through the insertion of a concentric needle electrode. The activity recorded on EMG is dependent to a large extent on the patient producing volitional contractions. EMG assessment can provide useful quantitative and qualitative information about Motor Unit Action Potentials (MUAPs). Spatiotemporal recruitment patterns of MUAPs in order to generate movements as well as the

presence of denervation and reinnervation can be characterized. In the first step of EMG examination, spontaneous, insertional, and volitional activity should be determined:

Insertional activity: The electrical activity recorded upon insertion of the needle electrode.

Spontaneous activity: This describes activity that manifests in denervated muscle such as fibrillations, positive sharp waves (attributable to spontaneously generated action potentials of individual muscle fibers), fasciculations (attributable to spontaneous discharges of individual MUs), and complex repetitive discharges (polyphasic action potentials that demonstrate uniform morphology and frequency of 5–100 Hz with abrupt onset and offset) (Fig. 2).

Once spontaneous and insertional activity has been assessed, the EMG examination can progress onto evaluating different morphological features of MUAPs

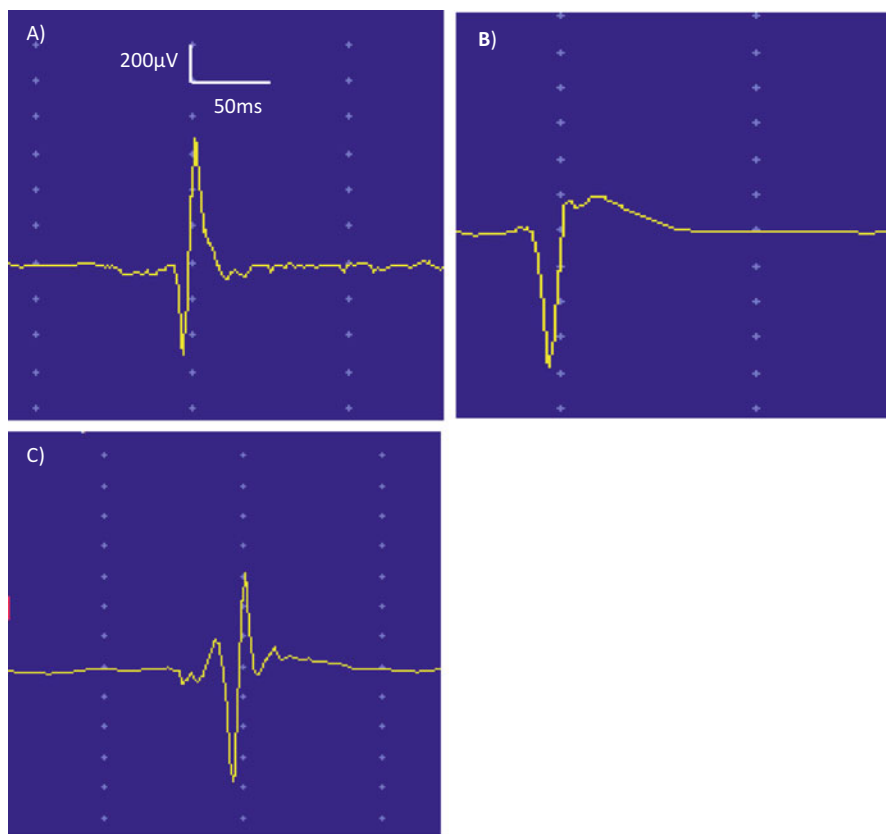


Fig. 2 Spontaneous activity recorded upon EMG examination of a denervated biceps muscle. (a) Fibrillations, (b) Positive sharp wave, (c) Fasciculation

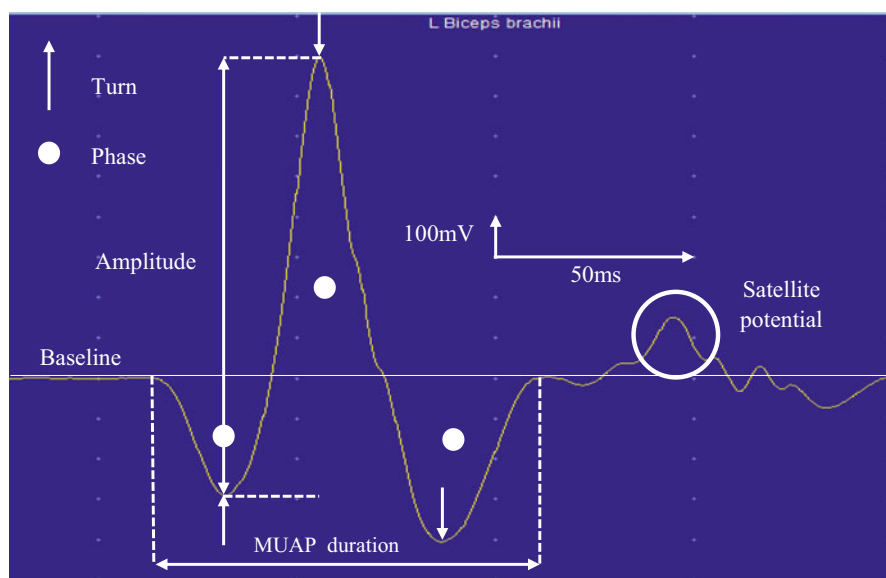


Fig. 3 A visual representation of a Motor Unit Action Potential (MUAP) recorded from a healthy participant and key morphological features

(Fig. 3) and their firing characteristics. This can provide highly sensitive information about the microanatomy of MUs which can be correlated with biological processes of muscle reinnervation. Specific features of the MUAP and their anatomical correlates are:

Amplitude: Measured from the maximum negative peak to the maximum positive peak. The magnitude of the amplitude has a positive correlation with the quantity and size of muscle fibers within 0.5 mm of the tip of the recording electrode (Buchthal et al. 1954a, b; Gydikov et al. 1980; Bromberg 1999).

Duration: Time interval between the initial shift of the MUAP away from baseline until its final return to baseline. The duration has a positive correlation with the number and size of muscle fibers that are within 2.5 mm of the tip of the recording electrode (Buchthal et al. 1954a, b; Gydikov et al. 1980; Bromberg 1999).

Area: Calculated as the area under the rectified waveform for its entire duration. This parameter has a positive correlation with the number and size of muscle fibers within 2 mm of the tip of the recording electrode (Buchthal et al. 1954a, b; Gydikov et al. 1980; Bromberg 1999).

Number of phases or turns: A phase represents the portion of the MUAP between initially deviating from baseline and returning to baseline. A turn describes a positive or negative peak throughout the duration of the MUAP. The number of phases and turns is positively correlated with the temporal dispersion of action potentials of muscle fibers within 1 mm of the tip of the recording electrode (Buchthal et al. 1954a, b; Gydikov et al. 1980; Bromberg 1999). The recording of

more than 4 phases is predictive of neurogenic muscle changes (Bromberg 1999; Takehara et al. 2004).

6.3 **Timing of Neurophysiological Assessment**

Results from electrodiagnostic studies that accurately characterize the presence or absence of neuropathology associated with PNI depend to a large extent on the timing of investigation. The temporal sequence of electrodiagnostic changes following nerve injury are not linear or immediately apparent following injury (Fig. 4). Moreover, the sequence of changes may be further confused by other factors such as the length of the distal stump with shorter stumps demonstrating an earlier onset of SNAP and/or CMAP abnormality following injury (Chaudhry and Cornblath 1992). Therefore, the timing of neurophysiological assessment following PNI is critical in order to reach an accurate diagnosis.

In the context of a nondegenerative nerve lesion (Tinel negative, Grade I injury), myelinated fibers may undergo a pathological process of focal demyelination which results in conduction slowing and/or block. Conduction slowing in isolation does not lead to clinical symptoms unlike conduction block which presents similarly to a degenerative lesion resulting in the loss of sensory and motor function. The CMAP recording can be used to distinguish between a lesion that has caused axonal loss and

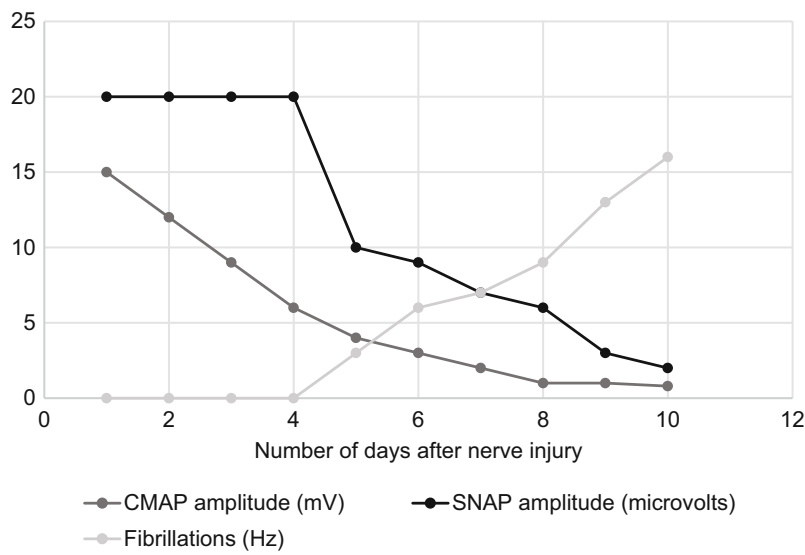


Fig. 4 A graphical representation of how neurophysiological parameters change in the context of a degenerative nerve lesion. Based on experimental findings and clinical observations following peripheral nerve injuries (Chaudhry and Cornblath 1992; Smith and Knight 2011)

conduction block; a CMAP that remains close to normal 7 days after the injury suggests that the target muscle has not undergone clinically significant axonal loss (Mallik and Weir 2005). Considering how the evoked responses of sensory and motor nerve potentials change upon recording proximal and distal to the lesion site can allow a diagnosis to be made with greater certainty. In conduction block, the CMAP is preserved upon stimulation distal to the injury site and reduced upon stimulation proximally or at the injury site. On the contrary, the CMAP is impaired across the entire course of the nerve where there has been axonal loss (Chaudhry and Cornblath 1992; Mallik and Weir 2005).

In a degenerative nerve lesion (Tinel positive, Grade II – V injuries), the CMAP generally remains stable for a few days, declining 3–5 days after the injury before becoming absent over the next 2–4 days (Fig. 4) reflecting the biological process of Wallerian degeneration (Chaudhry and Cornblath 1992). Changes in the sensory response tend to precede changes in the CMAP by 2–3 days (Chaudhry and Cornblath 1992). The time course of these changes is dependent to a large extent on the length of the distal stumps; the onset of abnormalities in NCS recordings is delayed in longer stumps (Miledi and Slater 1970).

Moving distally to the neuromuscular junction, no changes are evident until 18–20 h following axonal transection when there is complete failure of neuromuscular transmission (Miledi and Slater 1970). A reduction in the resting potential and increased sensitivity to acetylcholine are other changes that occur in response to a degenerative nerve lesion which are also dependent on the length of the distal stump (Smith and Thesleff 1976). Proliferation of acetylcholine receptors at sites beyond that of the motor endplate are largely responsible for these observations (Miledi and Slater 1970; Smith and Thesleff 1976; Smith and Knight 2011).

The appearance of abnormal spontaneous activity (fibrillations and sharp waves) occurs 1–4 weeks following injury (Chaudhry and Cornblath 1992; Smith and Knight 2011). This time interval is dependent on the distance between the injury site and MUs in the target muscle (Smith and Thesleff 1976; Chaudhry and Cornblath 1992). Asynchronous and spontaneous depolarization of the sarcolemma is responsible for the appearance of fibrillation potentials on EMG (Smith and Thesleff 1976). This is attributable to ultra-structural changes which result in increased sodium conductance which propagate along the course of the muscle fiber (Smith and Thesleff 1976; Smith and Knight 2011). The intensity of fibrillation potentials is associated with the severity of axonal damage; a greater extent of axonal damage leads to more intense fibrillation potentials which regress over time (Smith and Knight 2011; Wu et al. 2014).

In summary, considering the timing of changes in NCS and EMG recordings in the setting of different degrees of nerve injury, it is widely accepted that electrodiagnostic tests should be carried out between 8 and 10 days following the injury (Chaudhry and Cornblath 1992; Mallik and Weir 2005; Campbell 2008). This optimizes the chance for an accurate and timely diagnosis to inform the clinical management of PNI.

6.4 Localizing the Lesion

The menu of tests on offer to the clinical neurophysiologist allows the examiner to localize the nerve lesion with a high degree of accuracy. The lesion can be classified as pre-ganglionic or post-ganglionic by examining SNAPs. In nerve injuries proximal to the dorsal root ganglion (DRG), SNAPs will be preserved as the sensory nerve cell bodies remain intact within the ganglion. This will be accompanied by anesthesia in the dermatomal region together with impaired motor action potentials. In post-ganglionic lesions, the SNAP will be reduced owing to Wallerian degeneration in post-ganglionic sensory fibers (Mansukhani 2013).

6.5 Determining the Extent of Neural Injury Using Electro-Diagnostic Testing

Further interrogation of features apparent on NCS and EMG recordings following neural injuries can be useful in further stratifying nerve injuries according to their severity.

6.6 Conduction Block/Neurapraxia

Clinically, conduction block manifests as acute weakness of the limb. Motor NCS demonstrates a CMAP area/amplitude recorded proximal to the nerve lesion is reduced by at least 20% (usually >50%) when compared with the CMAP recorded distal to the nerve lesion. A focal reduction in conduction velocity is also often reported across the site of the nerve lesion (Mallik and Weir 2005). The clinical and neurophysiological abnormalities completely reverse upon remyelination (Menorca et al. 2013). The time to recovery can vary greatly with some clinical studies reporting the reversal of motor paralysis after time periods as long as 6 months although most cases resolve within 3 months following injury (Dumitru et al. 2002; Campbell 2008). EMG markers of denervation such as spontaneous activity are not found in neurapraxic injury since no degenerative changes in the muscle occur. However, this prolongs electrophysiological diagnosis since changes in EMG recordings may not occur for a period of up to 2 weeks following muscle denervation, therefore it is not possible to distinguish between conduction block and a more severe axonal degenerative lesion during this time period (Chaudhry and Cornblath 1992).

6.7 Axonotmesis

In trauma which has resulted in damage of intraneural structures and axonal degeneration, NCS can give useful information that can assist in the stratification of the severity of the lesion. The disconnected distal segment survives for 4–7 days as

Wallerian degeneration ensues along a proximal-distal gradient (Chaudhry and Cornblath 1992). As a result, the excitability of the distal stump progressively declines. After this time period, stimulation distal to the nerve lesion results in an absent CMAP. Therefore, the CMAP is initially small or absent proximal to the nerve lesion and preserved distal to the lesion. By the second week of injury, CMAP and SNAP show significant reductions in amplitude distal to the site of injury (Chaudhry and Cornblath 1992). In addition, EMG examination may reveal fibrillations and positive sharp waves at this time point (Chaudhry and Cornblath 1992; Campbell 2008; Smith and Knight 2011). The time taken for these changes on NCS and EMG examination is largely dependent on the length of the distal stump (Smith and Thesleff 1976).

6.8 Neurotmesis

In neurotmesis, the anatomical disorganization is so severe that spontaneous regeneration is impossible. After a period of 2 weeks following injury, distinctions between neurotmesis, axonotmesis, and lesser degrees of injury are not possible (Chaudhry and Cornblath 1992). The diagnosis of neurotmesis is only possible with direct visualization of the nerve but may be suspected in cases of open injury where penetrating trauma may have caused extensive neural damage. For these reasons, early surgical exploration is recommended (chapter ► [“Surgical Techniques in Nerve Repair”](#)) in order to optimize functional outcomes for the patient (Campbell 2008). A distinction must be made in the case of suspected iatrogenic nerve injury; early EMG examination within 2–3 days of trauma is recommended since the presence of volitional MUs is predictive of neural continuity despite the presence of clinical signs that may suggest otherwise (Aminoff 2004).

6.9 Intraoperative Neurophysiology

A number of indications as determined by clinical examination and/or changes in electrodiagnostic tests for surgical exploration or repair of injured nerves exist (Smith and Knight 2011) (see chapter ► [“Surgical Techniques in Nerve Repair”](#) for more details):

- Paralysis in the distribution of the injured nerve
- Closed injury with surrounding tissue damage
- Open injury that warrants external reduction and internal fixation
- Nerve injuries that copresent with arterial damage
- Traction injuries to the brachial plexus
- Nerve function that is deteriorating clinically and neurophysiologically after initial diagnosis
- An absence of neurological improvement on clinical examination and electrodiagnostic tests

- Failure of conduction block to improve 6 weeks after injury
- Persistent pain or neuroma formation

The use of neurophysiology intraoperatively has become an indispensable tool for the reconstructive nerve surgeon. A number of neurophysiological techniques have been devised to assist the surgeon in dissection, to isolate the injured region, protect against iatrogenic neural injuries, as well as monitor the function of sensory and motor nerves and their more proximal pathways. Nerve action potentials (NAPs), somatosensory, and evoked potentials, as well as motor evoked potentials (MEPs) are the most widely reported techniques employed by reconstructive nerve surgeons (Lee et al. 2004; Kline 2012).

Somatosensory evoked potentials (SSEPs) and MEPs can assist in determining the continuity of the corticospinal and somatosensory tracts which, by extension, assists the surgeon in localizing the anatomical site of the nerve lesion (Lee et al. 2004; Kline 2012).

SSEPs have become one of the most commonly deployed modalities of intraoperative neurophysiological investigation of supraclavicular brachial plexus lesions (Slimp 2000; Kline 2012). Recording the SSEP involves transcutaneous stimulation of the peripheral nerve. The action potential propagates along the course of the nerve to the dorsal root where it then ascends the dorsal columns in the spinal cord. The dorsal columns synapse at the cervicomedullary junction and decussate as the fibers ascend on to the thalamus and finally to the somatosensory cortex. The SSEP can therefore be recorded at the different sites along this anatomical course (typically the cervical spine and the scalp) to help isolate the location of the nerve lesion. The presence of a central response suggests that the dorsal root is in continuity but it should be noted that only a few hundred fibers need to be present to enable a response to be recorded in animal models (Zhao et al. 1993; Kline 2012).

MEPs are becoming increasingly utilized to determine the integrity of the descending corticospinal tract and ventral root (Lee et al. 2004; Sutter et al. 2007; Kline 2012). The motor cortex is stimulated by transcranial electrical stimulation (TES) (through the scalp). The electrical activity travels down the spinal cord and synapses onto the anterior horn cell where the action potential then propagates along the motor nerve to the neuromuscular junction where it causes depolarization of the muscle which can be recorded using EMG. The presence of an MEP indicates the presence of either continuity or reinnervation of the target muscle. However, it does not prove continuity across the roots; for example, where there is discontinuity in one root (such as C5) an MEP could still be detected in the muscle (such as biceps) due to innervation by an alternative root (such as C6). Moreover, the presence of an MEP is not necessarily predictive of functional muscle reinnervation.

Once the integrity of the ascending spinothalamic and descending corticospinal tracts has been measured, attention can be focused onto evaluating changes in the PNS as a result of neural trauma.

Assessments of the more distal component of the PNS such as free running EMG can allow more accurate localization and characterization of the nerve lesion. Free running and evoked EMG can be used to monitor the function of muscles or motor

roots determined to be at risk during surgery. This can be helpful in determining the site of the nerve lesion and its severity. For stimulated EMG, bipolar electrodes are preferred as this minimizes the dispersion of current to adjacent nerves (Robert et al. 1995); this is particularly pertinent in brachial plexus surgery.

NAPs across traumatic lesions can provide rapid and reliable information about the functional status of a nerve at the time of surgery. Areas of nerve dysfunction can be mapped and, through assessing segments of nerve distal to these areas of injury, it is possible identify if this lesion is degenerative (with no signal evident distal to the lesion) or a conduction block (intact conduction in segments of the nerve distal to the lesion).

Stimulation is usually done proximally and recording distal in order to minimize artifacts from MUAPs. Where a nerve lesion has been isolated to the post-ganglionic region, the presence of a NAP is predictive of several thousand intact axons and is correlated with good functional recovery (Kline and Happel 1993). In an avulsion lesion, the intact neurons of the DRG will be conducting NAPs but are not functional as they are disconnected from the CNS. Thus, it is important to consider the mechanism and nature of each injury and relate this to the neurophysiologic findings in pre-, intra-, and postoperative situations.

Together, these intraoperative neurophysiological assessments can assist the surgeon in determining the severity and location of the nerve lesion. This informs what, if any, surgical intervention is necessary.

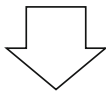
6.10 Electrophysiological Correlates of the Recovery of Motor Units

The rate of regeneration is around 1 mm/day across all somatic nerves (Seddon et al. 1943). Depending on the severity of the injury, nerve repair is thought to occur through two main mechanisms; collateral sprouting and neural regeneration along the course of the nerve fibers (Chen et al. 2007; Menorca et al. 2013). Optimal functional recovery depends on a sufficient quantity and quality of axons reestablishing functional connections with the target muscle within 1 year following injury (Kobayashi et al. 1997; Tung and Mackinnon 2010). After this time period, molecular and cellular changes in the proximal and distal stump as well as within the muscle are thought to make the environment increasingly antagonistic to successful regeneration (Wu et al. 2014; Jessen and Mirsky 2019). For these reasons, it is pertinent to carefully monitor patients for neurophysiological signs of reinnervation to inform the surgical management of patients to maximize functional recovery.

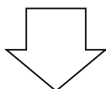
Muscle denervation is characterized by abnormal spontaneous activity (fibrillations and positive sharp waves) upon EMG assessment (Smith and Knight 2011). The intensity of this activity recedes as neural regeneration ensues and function is recovered although these changes are not easily quantifiable (Campbell 2008; Smith and Knight 2011). A number of other changes evident on NCS and EMG recordings correlate with early, ongoing, and late reinnervation (Fig. 5).

Denervation

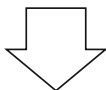
Spontaneous activity: fibrillations and sharp waves are evident in *acutely* denervated muscle.

**Early muscle renervation**

Motor Unit Potentials (MUPs) are normal with a longer duration. This is attributable to the larger innervation ratios of Motor Units (MUs).

**Ongoing muscle renervation**

The amplitude of MUPs is moderate, polyphasic and of longer duration. The firing characteristics are unstable secondary to unpredictable firing along the course of unmyelinated axonal sprouts.

**Chronically renervated muscle**

The amplitude of MUPs is increased and demonstrate a stable firing characteristics.

Fig. 5 A summary of changes in the characteristics of Motor Unit Action Potentials (MUAPs) after nerve injury and at different stages of muscle reinnervation (Smith and Knight 2011)

Neural regeneration is the only process through which functional recovery can be achieved following a complete nerve injury. As a result, neurophysiological monitoring of regeneration is notoriously challenging until functional neuromuscular junctions have been reestablished. The earliest signs of reinnervation are a low number of small unstable MUAPs known as nascent units which can be elicited by volitional effort or evoked potentials distal to the injury site (Smith and Knight 2011) (Fig. 5). A key characteristic of regenerating nerves is that they require a higher intensity stimulus in order to evoke a response, reduced conduction

velocities, and increased distal motor latencies until remyelination is complete. Recruitment is diminished and the frequency at which MUs are firing is disproportionately increased when compared with the number of MUs activated (Campbell 2008; Smith and Knight 2011). As the process of reinnervation continues, EMG examination reveals polyphasic MUAPs of longer duration with unstable firing due to the low safety margin at the neuromuscular junction as well as uncoordinated propagation of action potentials along unmyelinated fibers (Fig. 5) (Campbell 2008). In the later stages of muscle reinnervation, the size of the evoked CMAP increases and conduction velocities regress back towards pre-injury levels (Campbell 2008; Wu et al. 2014). MUAPs on EMG have larger amplitudes with stable transmission (Fig. 5).

Functional recovery following incomplete nerve injuries that have led to axonal degeneration incorporates a number of biological processes. Nerve fibers that have been spared injury undergo collateral sprouting and reinnervate adjacent muscle fibers (Hoffman 1950; Wu et al. 2014; Navarro 2016). The result are large MUs that have increased duration, amplitude, territory, and fiber density (Wu et al. 2014) (Fig. 5). Regeneration of axons that have been injured soon follows (Smith and Knight 2011). The quantity of reinnervation may be determined by neurophysiological measures such as quantitative MUAP analysis and/or Motor Unit Number Estimation (MUNE).

6.11 Quantitative MUAP Analysis

EMG recordings allow analysis of the morphological features of MUAPs which have demonstrated correlations with anatomy and neuropathological processes (Krarup et al. 2016). To this end, quantitative analysis of morphological features of MUAPs have been extensively studied to characterize MU remodeling during a number of different neurological diseases (Stalberg and Falck 1997; Flasar et al. 2017). The concentric needle electrode is reasonably selective and allows examination of the amplitude and area of MUAPs from muscle fibers within 500 μm and 200 μm of the tip of the electrode respectively (Buchthal et al. 1954a, b; Gydikov et al. 1980; Bromberg 1999; Takehara et al. 2004). This is particularly useful in assessing the recovery of MUs; it has been shown that changes of MUAP area, duration, and amplitude are highly sensitive indicators of early muscle reinnervation in pathologies such as Amyotrophic Lateral Sclerosis (ALS) (Joyce and Carter 2013). More recently, quantification of changes in the amplitude and duration of MUAPs have demonstrated close associations with reinnervation of facial muscles in a surgical model of hypoglossal facial nerve jump suture (Flasar et al. 2017).

Quantitative MUAP analysis does not provide information about the number of recovering functional MUs. Therefore, little or no association between quantitative MUAP analysis and functional outcomes has been widely reported. It follows that in order to foster the translation of new therapeutics for the treatment of PNI and to

improve clinical management, there is a need for measures that demonstrate improved relationships between the biological process that underpin nerve regeneration and the recovery of functional MUs.

6.12 Motor Unit Number Estimation (MUNE)

MUNE is a neurophysiological test that was first coined by McCommas to estimate the total number of functional MUs innervating a muscle (McCommas et al. 1971). MUNE is based on the phenomenon that it is possible to observe an increase in the amplitude of the response of a muscle with an increasing proximal nerve stimulus (McCommas et al. 1971). If the intensity of the stimulus is incrementally increased, it is possible to recruit individual MUs (McCommas et al. 1971). Dividing the CMAP by the mean stimulus intensity required to recruit an additional MU yields an estimate of the total MUs innervating the muscle (McCommas et al. 1971). Therefore, it is a technique that has been applied to study the dynamics of a number of different neurological pathologies that lead to the denervation of muscle (Gooch and Harati 2000; Lawson et al. 2003; Gooch et al. 2014).

A number of different methods have been developed since this original method of “incremental stimulation” first proposed by McCommas (de Carvalho et al. 2018). However, all techniques are based on the same principle of obtaining a CMAP and sample of Single Motor Unit Potentials (SMUPs) but can be differentiated by the way in which they record SMUPs. A recognized limitation of incremental stimulation is the phenomenon of alternation (Brown and Milner-Brown 1976; Kadrie et al. 1976). Alternation is where 2 MUs have as similar activation threshold and their individual responsiveness to the same stimulus intensity varies. This is particularly pertinent in the context of muscle reinnervation where the MU pool is more homogeneous with higher innervation ratios (Burnett and Zager 2004; Wu et al. 2014; Faroni et al. 2015). This led to the development of the Multipoint Stimulation (MPS) technique which overcomes this limitation by stimulating the nerve of interest at multiple sites along the anatomical course until at least 10 SMUPs are sampled (Brown and Milner-Brown 1976; Kadrie et al. 1976). However, this method is time consuming therefore a modified version that combined incremental stimulation and MPS was developed; the investigator stimulated 3 MUs from three different stimulation sites along the course of the nerve to obtain a sample of SMUPs.

Using stimulation to obtain a sample of SMUPs is not appropriate in proximal nerves. Due to anatomical reasons, it is often not possible to stimulate at a sufficient number of sites to obtain a representative sample of SMUPs. The digitalization of EMG recordings drove a number of advancements in MUNE which sought to circumvent these challenges. The spike triggered averaging (STA) technique involves inserting a concentric needle electrode into the muscle of interest to record EMG signals (Bromberg and Abrams 1995; Power et al. 2012; Gooch et al. 2014). An amplitude trigger is used to time lock and average signals from a single MU which are concurrently recorded using a surface electrode. Different MUs are recruited by isometric contraction of the muscle as well as sampling different

locations and depths of the muscle with the electrode to record a representative sample of SMUPs (Bromberg and Abrams 1995; Power et al. 2012; Gooch et al. 2014). The CMAP is divided by the averaged amplitude of SMUPs to obtain MUNE (Bromberg and Abrams 1995; Power et al. 2012). The negative peak area may also be determined for the CMAP and the SMUP for the MUNE calculation. Using this measure overcomes negative phase cancellation (Bromberg 2007) which is particularly prevalent when measuring reinnervated muscle where there is proliferation of acetylcholine receptors (Adams et al. 1995; Ma et al. 2007; Wu et al. 2014). However, a limitation of this method is the issue of patient tolerance to application of the needle electrode. This is of particular importance when multiple measurements are required to study the dynamics of different neurological pathologies.

Efforts have been subsequently made to allow reliable recording of MUNE from proximal muscles while also improving patient tolerance. This has informed further developments in techniques to obtain MUNE such as automated incremental stimulation (Galea et al. 2001), F Response method (Wang and Delwaide 1995), statistical method (Daube 1995), MUNIX (Motor Unit Index) (Nandedkar et al. 2004) and CMAP scanning (Jacobsen et al. 2018). However, the latter two methods have gained most widespread acceptance as the methods that demonstrate the highest repeatability and patient tolerance (de Carvalho et al. 2018).

In the MUNIX method, the CMAP is recorded by manipulating the location of the electrode to obtain the signal with the amplitude of the greatest magnitude. The subject is then requested to exert different grades of isometric contraction while the surface EMG interference pattern (SIP) is recorded. A mathematical model is then applied to determine the area of the CMAP and SIP which is used to calculate a motor unit index (Nandedkar et al. 2004). A drawback of this method is that the software required is expensive and this has limited more widespread clinical use.

The CMAP scan method involves increasing the stimulus in small increments and recording the evoked response from the target muscle. The intensity is increased to supramaximal levels in approximately 500 steps. The differences between each of these successive steps is then quantified after being sorted into an ascending or descending order. The number of largest differences that equate to 50% of the CMAP amplitude is named D-50. In the context of muscle reinnervation, this index reduces due to larger innervation ratios. A mathematical model can then be applied to this data to calculate MUNE which is available commercially. This model simulates the scanning method and “fits” it to the recorded results which reduces preferential detection of small and/or large MUs (Jacobsen et al. 2018).

Despite the wealth of advancements that have been made in developing methods to obtain MUNE, there remain a number of limitations. Firstly, MUNE is primarily dependent on the recording of a valid CMAP which is known to show little relationship with muscle size suggesting that it does not provide an accurate representation of the number of MUs (Nandedkar and Barkhaus 2007; de Carvalho et al. 2018). Moreover, it has been shown that muscle fibers that are located deeper than 15–20 mm deep to the recording surface electrodes are not reflected in the CMAP a limitation that also extends to the surface recording of MUPs (Barkhaus and Nandedkar 1994; Nandedkar and Barkhaus 2007). Thirdly, in the context of

reinnervated muscle where the motor endplate zone is more diffuse due to proliferation of acetylcholine receptors, identifying an optimal site for recording reliable CMAP recordings is more challenging (Wu et al. 2014; de Carvalho et al. 2018). For these reasons, MUNE remains only an estimate and further work is required to understand what relationship MUNE has to biological correlates of neural regeneration.

Despite these limitations, one of the main benefits of MUNE is good reliability irrespective of which method is used to derive it (Gooch and Harati 2000; Bromberg 2007; Gooch et al. 2014). Moreover, MUNE provides a unique assessment of the number of functioning MUs innervating a muscle that complements traditional EMG assessment. For these reasons, MUNE is favored for quantifying disease progression and has been employed as a primary outcome in trials that have looked at denervating pathologies such as ALS and Spinal Muscle Atrophy (SMA) (Gooch and Harati 2000; Bromberg 2007; Gooch et al. 2014). However, application of MUNE in the inverse physiology, muscle reinnervation, is awaited perhaps due to limited discussion in the surgical literature. This will be key in aiding the translation of therapies that have demonstrated promising outcomes in preclinical models of nerve injury.

6.13 Electrophysiological Correlates of Sensory Function

The regeneration of sensory nerves is closely related to the regeneration of MUs; the return of sensory potentials is widely reported at the same time as the appearance of a low number of unstable MUPs. The return of recordable SNAPs is thought to predict the presence of thousands of moderate-diameter sensory nerves. In animal models of PNI, this correlates with clinical recovery of sensory function (Smith and Knight 2011). The recovery of sensory nerves can also be clinically monitored through the Tinel sign (Macdonald 1918).

Autonomic deficits following PNI should also be considered and included in clinical evaluations. Post-ganglionic sympathetic fibers which traverse in close relationship with the nerve trunk are frequently damaged following PNI leading to loss of sweating, vasomotor tone, as well as a reduction in nail growth.

There are a number of neurophysiological quantitative sensory tests that can help determine which fibers have been most severely affected and the extent of damage or recovery.

6.14 Quantitative Sensory Testing

Quantitative sensory testing can quantify the recovery of a number of different components of the sensory and autonomic nervous system.

The sympathetic skin test involves transducing a current between two points on the skin. This stimulates the eccrine sweat glands. The fluid secreted onto the skin surface leads to an increase in resistance between the two points on the skin. This can

provide some indication as to the functioning the sympathetic system (Smith and Knight 2011).

A similar test is the sudomotor axon reflex test (QSART). This test involves the application of iontophoresed acetylcholine which antidromically stimulates the post-ganglionic sympathetic fibers. This leads to the release of acetylcholine orthodromically which results in sweat secretion. This measure can be quantified and has demonstrated high reliability (Smith and Knight 2011).

The recovery of larger myelinated A delta (cold threshold) and unmyelinated C (heat threshold) fibers can be determined using a psychophysical test known as thermal thresholds. By measuring the threshold for temperature sensation and applying the “method of limits” algorithm (Smith and Knight 2011), the recovery of these fibers can be monitored. The “method of limits” method involves applying a thermode to the skin and the temperature of the thermode is incrementally increase until the patient can feel a warm sensation at which point she or he depresses a button. This is defined as the perception threshold. The thermode is then cooled until the participant detects a cool sensation which is termed the disappearance threshold. A number of different cycles have been proposed from which a thermal threshold can be calculated (Yarnitsky and Sprecher 1994; Hilz et al. 1998; Chong and Cros 2004).

A number of different methods also exist to detect and quantify vibration sense using the “method of limits” (Chong and Cros 2004). In this test, the patient is asked to depress a button as soon as she or he can perceive vibration during incremental increases in vibration intensity (the perception threshold). Similarly, the patient is asked when she or he can no longer detect the vibration during incremental decreases in the intensity of the vibration (disappearance threshold) (Chong and Cros 2004).

6.15 Electrophysiological Correlates of the Response of the CNS to PNI

There are a number of changes in the CNS following PNI such as remodeling of the spinal cord. These biological and anatomical changes are associated with a change in crossed spinal reflexes such as the H-reflex (Valero-Cabré and Navarro 2001). These reflexes are not widely used in the evaluation of PNI.

In summary, neurophysiological assessments of PNI represent an indispensable tool in determining the location and extent of damage to the PNS. By extension, these tests can provide some indication to what the likely recovery will be. However, sensitive and responsive tests that quantify the biological recovery of MUs have not been well studied in models of nerve injury. This, along with understanding what relationship this has with functional recovery will be key to developing valid outcome measures.

Magnetic Resonance Imaging (MRI) and Ultrasound are other modalities which are indispensable in imaging PNI. Recent advancements have identified opportunities to identify changes associated with the biological processes of nerve regeneration.

7 Imaging of Peripheral Nerve Injuries

The mainstay for evaluation of PNI remains clinical examination with supporting NCS and EMG. However, a major restriction associated with these tests is that neurophysiological techniques cannot provide morphological details or demonstrate alterations within the nerve or in adjacent tissues that may have been affected by the injury. In addition, focal lesions are not amenable to neurophysiological evaluation (Koltzenburg and Bendszus 2004). While imaging is considered invaluable in the work up of patients with disorders of the CNS (Alvarez-Linera 2008; Murphy and Koh 2010; Zivadinov et al. 2017), widespread application for disorders of peripheral nerves is awaited (Rayner et al. 2019; Wilcox et al. 2020). This is despite recent studies demonstrating changes in MRI associated with damaged nerve and skeletal muscle (Bendszus et al. 2002, 2003; Koltzenburg and Bendszus 2004; Simon et al. 2016; Kakkar et al. 2018). These findings may provide opportunities to develop sensitive, responsive, and valid outcome measures through which the biological process of nerve regeneration can be monitored and measured.

In addition, there are a number of practical benefits to imaging; unlike needle electromyography, imaging does not require the active cooperation of the patient and is painless. MRI provides a permanent document of the topographical area studied. Unlike ultrasonography, this does not depend on the experience of the examiner to relocate the area of interest. Focusing on the clinical benefits, there are a number of scenarios which could warrant the use of imaging rather than neurophysiology. For example, in patients who are on anticoagulation or after tissue damage following a traumatic injury where the nerve or muscle may be difficult to reach due to anatomical reasons. In these circumstances, imaging may offer a viable alternative or valuable adjunct to the evaluation of PNI.

Given the potential benefits imaging may have in characterizing neural injuries, this section explains how different imaging modalities may be useful in detecting and quantifying changes associated with traumatic PNI.

7.1 MRI Findings in Injured Nerves

A normal nerve is found to be isointense or slightly hyperintense on T2-weighted (T2-w) images compared to surrounding muscle. Therefore, it is often challenging to discriminate between muscles and nerves. However, in the context of a degenerative nerve lesion (axonotmesis or neurotmesis), damaged nerves demonstrate a significant increase in T2- relaxation time (they appear bright on T2-w images). In animal models, it has been shown that changes in the signal intensity are apparent within 24 h after insult and demonstrate a proximo-distal gradient starting at the lesion site (Kullmer et al. 1998; Bendszus et al. 2002; Koltzenburg and Bendszus 2004; Holzgrefe et al. 2019). If successful nerve regeneration occurs, these signal changes regress back to normal levels over several weeks and are closely associated with the histological and electrophysiological markers of muscle reinnervation (Kikuchi et al. 2003; Wessig et al. 2004). Together, this evidence suggests that there is a close association between MR signal

changes and the biological process of peripheral nerve regeneration. Comparative studies in human models of regeneration are needed to realize the benefits this method could have in determining axonal injury, likely prognosis and what relationship these changes have with functional measures of recovery.

A number of reasons for the hyperintense signal on T2-w images of injured nerves have been posited; loss of myelin, an increase in the extracellular space, axonal loss, as well as disruption of the axoplasmic transport system are all mechanisms that have been suggested to be at least partially responsible (Titelbaum et al. 1989; Does and Snyder 1996; Stanisz et al. 2001; Cudlip et al. 2002).

MRI has also emerged as a sensitive tool for the diagnosis of neurapraxia (nondegenerative lesions) such as peroneal nerve compression at the fibular head (Koltzenburg and Bendszus 2004), thoracic outlet syndrome (Panegyres et al. 1993), and cervical radiculopathy (Dailey et al. 1996).

7.2 MRI Findings in Denervated Muscle

Normal muscle tissue can be visualized as an intermediate signal on T1-weighted (T1-w) and T2-w sequences. Clinical reports demonstrate that acutely (<1 month) and subacutely (1–6 months) denervated muscle manifest hyperintense signals on MRI sequences that are fluid sensitive including short tau inversion recovery (STIR) and turbo inversion recovery magnitude (TIRM) while retaining normal intensity on T1-w images (Polak et al. 1988; Kullmer et al. 1998; Bendszus et al. 2002; Aagaard et al. 2003). In clinical studies, changes in signal intensity have been reported within 4 days following the injury. In other cases, these changes may evolve over several weeks and persist for several months in denervated muscle (Kamath et al. 2008). As the muscle becomes more chronically denervated (>6 months), there is further atrophy and infiltration of fat, features that can be seen on T1-w and CT images (Clague et al. 1995). The abnormalities in MR signal have been reported to regress back to normal levels within 4–6 weeks following the onset of reinnervation (Viddeleer et al. 2012). Other studies have shown that abnormalities in T2 relaxation time reverse within 10 weeks of the beginning of nerve regeneration (Kamath et al. 2008). Comparable findings have been reported in animal paradigms of PNI with a statistically significant increase in signal on T2-w images of denervated muscles within 48 h following injury which continues to increase for a further 2–4 weeks (Kamath et al. 2008). If nerve regeneration is successful, the signal regresses back towards normal levels 6–8 weeks post-injury (Kamath et al. 2008). If regeneration fails, muscle atrophy ensues which is accompanied with persistent hyperintense signals on MRI (Kamath et al. 2008).

Focusing on the relationship of MR signal changes with EMG recordings, it has been reported that signal increases on T2-w images of denervated muscle may precede the onset of spontaneous activity in animal and human models of regeneration (Fig. 6) (Wessig et al. 2004). Moreover, the decline of hyperintense signals may also precede the regression of spontaneous activity (Bendszus et al. 2003; Wessig et al. 2004; Simon et al. 2016). There is also some contrary evidence which suggests that prolongation of the T2 relaxation time and gadolinium enhancement of denervated muscle occur in

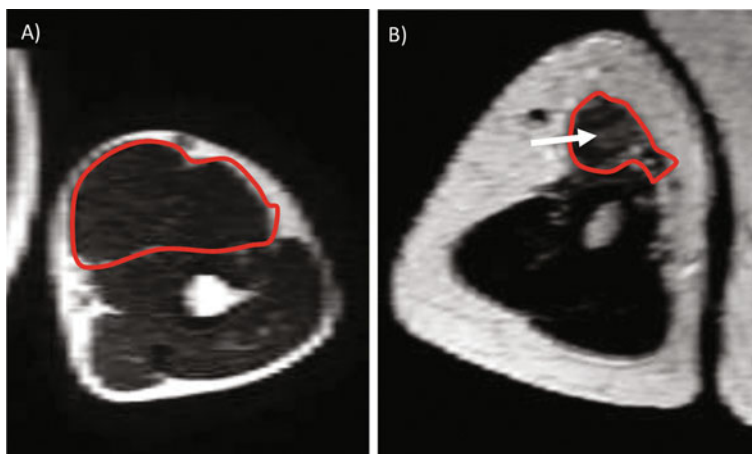


Fig. 6 T2-weighted MRI scans of uninjured and nerve injured biceps muscles from patient who sustained C5/6 Avulsion. **(a)** Uninjured biceps muscle (uninjured contralateral arm) outlined in red. **(b)** Subacutely denervated biceps muscle (3 months following injury) demonstrating increased signal (arrow) and muscle atrophy of biceps muscle (outlined in red) compared to **(a)**

parallel to the development of spontaneous activity on EMG (Bendszus and Koltzenburg 2001; Koltzenburg and Bendszus 2004). Together, this warrants further exploration of the ability of signal changes to predict the likely functional recovery and how they relate to neurophysiological parameters.

The cellular and molecular features that underpin the changes in signal intensities on MRI scans of denervated muscle have not been elucidated. It has been postulated that the signal changes on MR sequences that are fluid sensitive are attributable to an increase in capillary density and/or extracellular volume (Koltzenburg and Bendszus 2004; Bendszus and Stoll 2005). In the subacute denervation period, it is thought that muscle has an increase in the relative amount of fat and extracellular water volume/capillary density (Carlson 2014; Wu et al. 2014). It is posited that this increase in fat is offset by the increase in extracellular space/capillary density which means that there is no overall change in the T1-relaxation time. For this reason, acutely denervated muscle may be better detected on water sensitive MR sequences. This theory is supported by experimental findings that have shown increased uptake of the extracellular contrast agent Gadolinium diethylenetriaminepentaacetate (Gd-DPTA) by denervated muscle on T1-w images which closely parallels changes in T2 relaxation time (Bendszus and Koltzenburg 2001).

7.3 Muscle Volume Changes in Denervated Muscle and the Application of MRI

The past decade has seen the advent of volumetric assessment from imaging analysis (CT, MRI and Ultrasound). Changes in volumes of organs within the CNS have been utilized as a biomarker to predict the onset and likely prognosis of a number of

neurodegenerative conditions (Shen et al. 2011; Maglioni et al. 2018; Petrone et al. 2018; Mitolo et al. 2019). However, application in disorders of the PNS is awaited.

Following nerve transection, it has been shown that rat muscle wet weight demonstrates an initial dramatic decrease in wet weight following denervation. This stabilizes at 10–20% of the weight of the healthy, uninjured contralateral side 3–12 months following denervation. The recovery of wet weight can be determined by the size of the nerve gap, severity of nerve damage as well as the time interval between injury and surgical repair of the damaged nerve. The best recovery of wet weight is when nerve repair is completed within 1 month following denervation with wet weight recovering to 19–100% of uninjured contralateral side. When the delay is extended to 3 months or more, the recovery of wet weight is only 10–20% of the uninjured contralateral side (Wu et al. 2014).

These changes in muscle wet weight have significant clinical relevance in that they can theoretically be applied to predict the likely success or failure of surgical nerve repair (Fig. 6). However, it is impossible to measure muscle wet weight in humans. Therefore noninvasive imaging techniques such as MRI could theoretically be used to measure volume as a surrogate for wet weight. This could provide a quantitative correlate of neural regeneration which has the capacity to predict likely functional recovery in the setting of neural regeneration. A significant barrier that exists is a limited understanding of what relationship muscle volume has with objective and subjective measures of functional outcomes (Rayner et al. 2019; Wilcox et al. 2020). Moreover, a standardized model of human peripheral nerve regeneration through which these changes can be quantified is awaited.

7.4 MR Neurography

Recent decades have seen the advent of novel application of MRI techniques for diagnosing a number of PNS disorders (Dailey et al. 1997; Rose et al. 2010; Baltodano et al. 2014). In the 1990s, magnetic resonance neurography (MRN) was developed which involves the application of a set of MR pulse sequences to distinguish nerves from the surrounding tissues. MRN involves the application of fat-saturated heavily T2-w sequences in order to distinguish the bright nerve signal from the adjacent fat tissue (Howe et al. 1992).

Normal nerves on high-resolution T1-w images appear round in shape and demonstrate a fascicular pattern. A hyperintense halo often surrounds the nerve which represents the epineural fat tissue. On fat saturated T2-w images, normal nerves appear isointense or marginally hyperintense compared to the surrounding muscle tissue (a thorough review of the appearance of normal nerves is available elsewhere) (Filler et al. 1993; Moser et al. 2009; Deroide et al. 2010).

Degenerative nerve lesions result in a hyperintense nerve signal in the distal stump that persists for months to years following the lesion (Filler et al. 1996; Kuntz et al. 1996; Dailey et al. 1997; Maravilla and Bowen 1998; Koltzenburg and Bendszus 2004). This differs from the hyperintense signal that is evident on MRI images where the T2 signal will regress to normal levels in a proximo-distal gradient once neural regeneration has commenced (Bendszus and Koltzenburg 2001;

Bendszus et al. 2002; Wessig et al. 2004). A further advantage of MRN is that neurotmesis can be directly visualized as discontinuity of the nerve of formation or a neuroma (Filler et al. 1993). This can have important implications on the surgical management of this complex pathology. A limitation of MRN is that signal intensities may return to normal levels in neurotmesis injuries (Koltzenburg and Bendszus 2004; Bendszus and Stoll 2005). This means that the clinician cannot distinguish between axonotmesis and neurotmesis. This has promoted the development of novel contrast agents to further characterize and evaluate the nerve lesion.

7.5 MR Contrast Agents

The advent of new contrast agents has enabled remarkable insights into the pathophysiology that underpins neural regeneration and has improved the specificity of MRN. However, no simple association between changes in signal intensity and nerve function has been detailed.

Nerve degeneration and regeneration has been visualized using gadolinium-based MR contrast agents such as gadofluorine M (Gf). This agent selectively accumulates and remains within nerve fibers that are undergoing Wallerian degeneration. This hyperintense signal was evident within 48 h following injury throughout the entirety of the nerve segment that was undergoing Wallerian degeneration. This abnormal hyperintense signal regressed back to normal levels along a proximo-distal gradient as neural regeneration ensued. However, in nerve fibers which had failed to regenerate, the signal persisted for several weeks after the injury (Bendszus and Koltzenburg 2001; Bendszus and Stoll 2005).

Reticuloendothelial contrast agents such as supramagnetic iron oxide (SPIO) particles have provided novel *in vivo*, real-time insights into the neuropathology that underpins neural regeneration (Bendszus and Stoll 2003). The SPIO particles were injected into the systemic circulation 24 h before MRI at different time points following injury (Bendszus and Stoll 2003). The SPIO particles are then rapidly phagocytosed by macrophages within the reticuloendothelial system located in reticular connective tissue. As a result, a proportion of macrophages become iron-labelled. Their accumulation within tissues can then be closely monitored through increases in signal on T2-w images (Bendszus and Stoll 2003; Bendszus and Stoll 2005). This has allowed imaging of biological events key to successful nerve repair such as Wallerian degeneration (Bendszus and Stoll 2003); a process that commences soon after injury and involves the infiltration of immune cells such as macrophages which clear myelin and other debris which are events thought to be critical in order to permit successful nerve regeneration (Fu and Gordon 1997; Faroni et al. 2015).

7.6 MRN to Quantify Axonal Diameter

MRN has been applied beyond changes in signal intensity. The advent of new sequences has enabled the quantification of axonal diameters (Kakkar et al. 2018). It has been shown that the diameter of axons can vary in health and disease (Cluskey and Ramsden

2001; Burnett and Zager 2004; Stassart et al. 2018). Following a nerve injury, axons demonstrate a reduced caliber (Burnett and Zager 2004; Sulaiman and Gordon 2013). As nerve regeneration ensues, the diameter of axons increase towards preinjury levels (Burnett and Zager 2004; Sulaiman and Gordon 2013). This change in diameter could theoretically be used as a quantitative marker of nerve regeneration.

The imaging of axon diameters using MRI has been made possible through the application of single diffusion encoding (SDE) spin echo sequences (Jespersen 2012). More recent studies have used oscillating spin gradient echo (OSGE) sequences which have wider applications in the clinical arena where nerve fibers may have an unknown or dispersed orientation (Parsons et al. 2006; Shemesh et al. 2015; Drobnjak et al. 2016; Jiang et al. 2016; Mercredi et al. 2017). The values for axonal diameter obtained using this method have shown excellent correlations with histological measurements of diameter (Kakkar et al. 2018). Together, this may provide a new sensitive and responsive measure of axonal regeneration.

7.7 Tractography

Peripheral nerves are highly anisotropic structures. Diffusion tensor imaging (DTI) with tractography exploits this feature of nerves to image them in healthy and disease (Jeon et al. 2018). Free water demonstrates random movement of hydrogen nuclei. In nerve tracts, the majority of water diffuses along the longitudinal course of nerves. Following a nerve injury, there is disruption to the microanatomy of the nerve. This permits the diffusion of a higher proportion of water molecules orthogonally to the course of the nerve trunk. Since the pattern of diffusion differs between healthy and injured nerves, this imaging technique can provide a wealth of information that is useful in characterizing the nerve lesion.

DTI measures this random diffusion of water and provides quantitative indices which can provide information about the structure and orientation of the nerve. These indices are known as eigenvalues ($\lambda_1, \lambda_2, \lambda_3$), eigenvectors (e_1, e_2, e_3), and fractional anisotropy (FA) (Li et al. 2013; Boyer et al. 2015; Gallagher et al. 2015; Heckel et al. 2015). Changes in these values are predictive of changes in the microanatomy of the nerve trunk (Morisaki et al. 2011; Li et al. 2013; Boyer et al. 2015; Gallagher et al. 2015; Heckel et al. 2015; Jeon et al. 2018).

The validation of DTI in evaluating nerve injury has been extensively studied; in particular, FA values demonstrate strong associations with histological markers of denervation and reinnervation (Morisaki et al. 2011; Gallagher et al. 2015; Heckel et al. 2015). Animal and human models of nerve injury have shown that FA values are lower in injured nerves when compared to uninjured nerves (Morisaki et al. 2011; Gallagher et al. 2015; Heckel et al. 2015). This has been attributed to lower quantities of myelin and anisotropic diffusion of water molecules (Morisaki et al. 2011; Gallagher et al. 2015; Heckel et al. 2015). DTI measurements can also assist in determining the stage of nerve regeneration; Wallerian degeneration has been associated with a specific set of changes as has the onset of neural regeneration and muscle reinnervation (Morisaki et al. 2011; Gallagher et al. 2015; Heckel et al. 2015).

7.8 Ultrasound

Ultrasonography (US) enables real-time *in vivo* visualization of peripheral nerves and the surrounding soft tissue with high spatial resolution of up to 400 μm using 18 MHz transducers (Visser 2006). In general, the highest available frequency should be used to image peripheral nerves. However, the higher the frequency, the lower the penetration to deeper tissues. Therefore, the use of 18 MHz transducers is recommended for the examination of superficial nerves while deeper nerves such as the Sciatic nerve indicate the use of 10–12 MHz transducers (Visser 2006; Simon et al. 2016). A normal nerve appears as hyperechoic neuronal fascicles and echogenic surrounding connective tissue. These features enable the differentiation of the nerve from hyperechoic structures surrounding the nerve such as tendons and muscles (Simon et al. 2016).

In the context of PNI, a number of features can be demonstrated on high-resolution ultrasonography such as axonal swelling, neuroma formation, as well as nerve discontinuity (Kullmer et al. 1998; Simon et al. 2016). Ultrasonography has the highest sensitivity for the detection of these features 72 h after trauma (Kullmer et al. 1998; Simon et al. 2016). Since ultrasonography allows rapid examination of long length of nerve in a single examination, it has been widely reported as the preferred first-line modality for the investigation of PNI (Tagliafico et al. 2010; Simon et al. 2016). Unlike neurophysiology, ultrasonography can distinguish between neurotmesis and severe axonotmesis long before reinnervation (Kullmer et al. 1998; Simon et al. 2016).

There has been much written on the sensitivity and specificity of ultrasound in the diagnosis of neuralgic amyotrophy (brachial neuritis), an inflammatory nerve injury which has recently been characterized as having a diagnostic torsional appearance on ultrasound (Abraham et al. 2016; Seror 2017; van Rosmalen et al. 2019).

While ultrasonography may provide a number of benefits for the management of PNI in the acute and subacute setting, there are a number of drawbacks to this technique; it is user dependent and contrast resolution is often disappointing (Ohana et al. 2014). As a result, ultrasonography provides a limited scope for the quantification and prospective evaluation of neuropathological processes underlying PNI when compared to MRI and neurophysiology (Holzgreffe et al. 2019).

8 Conclusions

The following issues must be addressed in order to develop valid, sensitive, and responsive clinical outcome measures that relate to functional outcomes following nerve repair.

Firstly, existing clinical assessments of the recovery of muscular function do not fully represent the lived experience of muscle reinnervation as reported by patients (Brown et al. 2018; Rayner et al. 2019; Wilcox et al. 2020). Recent qualitative and quantitative studies have refocused the attention of researchers to develop objective and subjective measures that better reflect the lived experience of muscle

reinnervation. This is discussed in greater detail in chapter ► [“Rehabilitation of Nerve Injuries.”](#)

Second, while neurophysiological assessments can provide important information about the site and extent of the injury, parameters that can quantify the degree of nerve regeneration such as MUNE have not been well documented in human models of regeneration. Moreover, evident on imaging modalities such as MRI and Ultrasound are thought to provide sensitive and responsive markers of nerve regeneration. Relating these changes to the recovery of muscular function in human models of nerve regeneration will be key to realizing the clinical benefits of these technologies.

This approach will have a number of benefits enabling the clinical translation of new treatments for PNI. In addition, the detection of subclinical biological changes will also allow clinical outcomes to be better predicted improving the clinical management of PNI patients.

References

- (1917) Nerve wounds symptomatology of peripheral nerve lesions caused by War Wounds. By J. Tinel, late Chef de Clinique at La Salpêtrière, with Preface by Professor Dejerine, translated by Fred Rothwell, revised and edited by Cecil A. Joll. Pp. xii + 317, with 323 illustrations. London Baillière, Tindal & Cox. 15s. net. *BJS* 5(19):517
- (1986) Classification of chronic pain. Descriptions of chronic pain syndromes and definitions of pain terms. Prepared by the International Association for the Study of Pain, Subcommittee on Taxonomy. *Pain Suppl* 3:S1-226
- Aggaard BD, Lazar DA, Lankerovich L, Andrus K, Hayes CE, Maravilla K, Kliot M (2003) High-resolution magnetic resonance imaging is a noninvasive method of observing injury and recovery in the peripheral nervous system. *Neurosurgery* 53(1):199–203; discussion 203–194
- Aberg M, Ljungberg C, Edin E, Jenmalm P, Millqvist H, Nordh E, Wiberg M (2007) Considerations in evaluating new treatment alternatives following peripheral nerve injuries: a prospective clinical study of methods used to investigate sensory, motor and functional recovery. *J Plast Reconstr Aesthet Surg* 60(2):103–113
- Abraham A, Izenberg A, Dodig D, Bril V, Breiner A (2016) Peripheral nerve ultrasound imaging shows enlargement of peripheral nerves outside the brachial plexus in neuralgic amyotrophy. *J Clin Neurophysiol* 33(5):e31–e33
- Adams L, Carlson BM, Henderson L, Goldman D (1995) Adaptation of nicotinic acetylcholine receptor, myogenin, and MRF4 gene expression to long-term muscle denervation. *J Cell Biol* 131(5):1341–1349
- Alvarez-Linera J (2008) 3T MRI: advances in brain imaging. *Eur J Radiol* 67(3):415–426
- Aminoff MJ (2004) Electrophysiologic testing for the diagnosis of peripheral nerve injuries. *Anesthesiology* 100(5):1298–1303
- Ashley Z, Sutherland H, Lanmuller H, Russold MF, Unger E, Bijak M, Mayr W, Boncompagni S, Protasi F, Salmons S, Jarvis JC (2007) Atrophy, but not necrosis, in rabbit skeletal muscle denervated for periods up to one year. *Am J Physiol Cell Physiol* 292(1):C440–C451
- Baltodano PA, Tong AJ, Chhabra A, Rosson GD (2014) The role of magnetic resonance neurography in the postoperative management of peripheral nerve injuries. *Neuroimag Clin N Am* 24(1):235–244
- Barkhaus PE, Nandedkar SD (1994) Recording characteristics of the surface EMG electrodes. *Muscle Nerve* 17(11):1317–1323
- Bendszus M, Koltzenburg M (2001) Visualization of denervated muscle by gadolinium-enhanced MRI. *Neurology* 57(9):1709–1711

- Bendszus M, Stoll G (2003) Caught in the act: in vivo mapping of macrophage infiltration in nerve injury by magnetic resonance imaging. *J Neurosci* 23(34):10892–10896
- Bendszus M, Stoll G (2005) Technology insight: visualizing peripheral nerve injury using MRI. *Nat Clin Pract Neurol* 1(1):45–53
- Bendszus M, Koltzenburg M, Wessig C, Solymosi L (2002) Sequential MR imaging of denervated muscle: experimental study. *AJNR Am J Neuroradiol* 23(8):1427–1431
- Bendszus M, Wessig C, Reiners K, Bartsch AJ, Solymosi L, Koltzenberg M (2003) MR imaging in the differential diagnosis of neurogenic foot drop. *AJNR Am J Neuroradiol* 24(7):1283–1289
- Birch R (2011) Pain. In: Birch R (ed) *Surgical disorders of the peripheral nerves*. Springer, London, pp 527–561
- Bostock H, Sears TA (1976) Continuous conduction in demyelinated mammalian nerve fibers. *Nature* 263(5580):786–787
- Bostock H, Sears TA, Sherratt RM (1981) The effects of 4-aminopyridine and tetraethylammonium ions on normal and demyelinated mammalian nerve fibres. *J Physiol* 313:301–315
- Boyd JG, Gordon T (2003) Glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor sustain the axonal regeneration of chronically axotomized motoneurons in vivo. *Exp Neurol* 183(2):610–619
- Boyer RB, Kelm ND, Riley DC, Sexton KW, Pollins AC, Shack RB, Dortch RD, Nanney LB, Does MD, Thayer WP (2015) 4.7-T diffusion tensor imaging of acute traumatic peripheral nerve injury. *Neurosurg Focus* 39(3):E9–E9
- Brattain K (2012) Analysis of the peripheral nerve repair market in the US. Magellan Medical Technology Consultants, Minneapolis
- Bromberg MB (1999) Electronic myoanatomic atlas for clinical electromyography muscle localization for needle insertion in clinical EMG CD-ROM. Electronic atlas of electromyographic waveforms, EMG on CD. Vol. II, Parts I–IV. *Muscle Nerve* 22(10):1468–1469
- Bromberg MB (2007) Updating motor unit number estimation (MUNE). *Clin Neurophysiol* 118(1):1–8
- Bromberg MB, Abrams JL (1995) Sources of error in the spike-triggered averaging method of motor unit number estimation (MUNE). *Muscle Nerve* 18(10):1139–1146
- Brown WF, Milner-Brown HS (1976) Some electrical properties of motor units and their effects on the methods of estimating motor unit numbers. *J Neurol Neurosurg Psychiatry* 39(3):249–257
- Brown H, Johnson K, Gilbert A, Quick TJ (2018) The lived experience of motor recovery of elbow flexion following Oberlin nerve transfer: a qualitative analysis. *Hand Therapy* 23(4):130–138
- Buchthal F, Guld C, Rosenfalck P (1954a) Action potential parameters in normal human muscle and their dependence on physical variables. *Acta Physiol Scand* 32(2-3):200–218
- Buchthal F, Pinell P, Rosenfalck P (1954b) Action potential parameters in normal human muscle and their physiological determinants. *Acta Physiol Scand* 32(2-3):219–229
- Burnett MG, Zager EL (2004) Pathophysiology of peripheral nerve injury: a brief review. *Neurosurg Focus* 16(5):E1
- Campbell WW (2008) Evaluation and management of peripheral nerve injury. *Clin Neurophysiol* 119(9):1951–1965
- Carlson BM (2014) The biology of long-term denervated skeletal muscle. *Eur J Trans Myol* 24(1):3293–3293
- Chammas M, Micallef JP, Prefaut C, Allieu Y (1997) Fatigue analysis of human reinnervated muscle after microsurgical nerve repair. *Clin Orthop Relat Res* 334:144–149
- Chassard M, Pham E, Comtet JJ (1993) Two-point discrimination tests versus functional sensory recovery in both median and ulnar nerve complete transections. *J Hand Surg Br Eur Vol* 18(6):790–796
- Chaudhry V, Comblath DR (1992) Wallerian degeneration in human nerves: serial electrophysiological studies. *Muscle Nerve* 15(6):687–693
- Chen Z-L, Yu W-M, Strickland S (2007) Peripheral regeneration. *Ann Rev Neurosci* 30:209–233
- Chong PST, Cros DP (2004) Technology literature review: quantitative sensory testing. *Muscle Nerve* 29(5):734–747

- Churchill JD, Arnold LL, Garraghty PE (2001) Somatotopic reorganization in the brainstem and thalamus following peripheral nerve injury in adult primates. *Brain Res* 910(1–2):142–152
- Ciaramitaro P, Mondelli M, Logullo F, Grimaldi S, Battiston B, Sard A, Scarinzi C, Migliaretti G, Faccani G, Cocito D (2010) Traumatic peripheral nerve injuries: epidemiological findings, neuropathic pain and quality of life in 158 patients. *J Peripher Nerv Syst* 15(2):120–127
- Clague JE, Roberts N, Gibson H, Edwards RH (1995) Muscle imaging in health and disease. *Neuromuscul Disord* 5(3):171–178
- Cluskey S, Ramsden DB (2001) Mechanisms of neurodegeneration in amyotrophic lateral sclerosis. *Mol Pathol* 54(6):386–392
- Cudlip SA, Howe FA, Griffiths JR, Bell BA (2002) Magnetic resonance neurography of peripheral nerve following experimental crush injury, and correlation with functional deficit. *J Neurosurg* 96(4):755–759
- Dailey AT, Tsuruda JS, Goodkin R, Haynor DR, Filler AG, Hayes CE, Maravilla KR, Kliot M (1996) Magnetic resonance neurography for cervical radiculopathy: a preliminary report. *Neurosurgery* 38(3):488–492. discussion 492
- Dailey AT, Tsuruda JS, Filler AG, Maravilla KR, Goodkin R, Kliot M (1997) Magnetic resonance neurography of peripheral nerve degeneration and regeneration. *Lancet* 350(9086):1221–1222
- Daube JR (1995) Estimating the number of motor units in a muscle. *J Clin Neurophysiol* 12(6):585–594
- de Carvalho M, Barkhaus PE, Nandedkar SD, Swash M (2018) Motor unit number estimation (MUNE): where are we now? *Clin Neurophysiol* 129(8):1507–1516
- Delgado DA, Lambert BS, Boutris N, McCulloch PC, Robbins AB, Moreno MR, Harris JD (2018) Validation of digital visual analog scale pain scoring with a traditional paper-based visual analog scale in adults. *J Am Acad Orthop Surg Global Res Rev* 2(3):e088–e088
- Dellon AL, Mackinnon SE (1988) Basic scientific and clinical applications of peripheral nerve regeneration. *Surg Ann* 20:59–100
- Deroide N, Bousson V, Lévy BI, Laredo JD, Kubis N (2010) L'imagerie du nerf et du muscle dans les atteintes nerveuses périphériques associée à l'électroneuromyographie: le couple idéal ? *La Revue de Médecine Interne* 31(4):287–294
- Does MD, Snyder RE (1996) Multiexponential T2 relaxation in degenerating peripheral nerve. *Magn Reson Med* 35(2):207–213
- Drobnjak I, Zhang H, Ianus A, Kaden E, Alexander DC (2016) PGSE, OGSE, and sensitivity to axon diameter in diffusion MRI: insight from a simulation study. *Magn Reson Med* 75(2):688–700
- Duff SV (2005) Impact of peripheral nerve injury on sensorimotor control. *J Hand Ther* 18(2):277–291
- Dumitru D, Zwarts MJ, Amato AA (2002) Peripheral nervous system's reaction to injury. In: *Electrodiagnostic medicine*. Hanley & Belfus, Philadelphia
- Faroni A, Mobasser SA, Kingham PJ, Reid AJ (2015) Peripheral nerve regeneration: experimental strategies and future perspectives. *Adv Drug Deliv Rev* 82–83:160–167
- Filler AG, Kliot M, Winn HR, Tsuruda JS, Hayes CE, Howe FA, Griffiths JR, Filler AG, Bell BA, Filler AG (1993) Magnetic resonance neurography. *Lancet* 341(8846):659–661
- Filler AG, Kliot M, Howe FA, Hayes CE, Saunders DE, Goodkin R, Bell BA, Winn HR, Griffiths JR, Tsuruda JS (1996) Application of magnetic resonance neurography in the evaluation of patients with peripheral nerve pathology. *J Neurosurg* 85(2):299–309
- Flasar J, Volk GF, Granitzka T, Geißler K, Irintchev A, Lehmann T, Guntinas-Lichius O (2017) Quantitative facial electromyography monitoring after hypoglossal-facial jump nerve suture. *Laryngoscope Investig Otolaryngol* 2(5):325–330
- Fu SY, Gordon T (1995) Contributing factors to poor functional recovery after delayed nerve repair: prolonged denervation. *J Neurosci* 15(5 Pt 2):3886–3895
- Fu SY, Gordon T (1997) The cellular and molecular basis of peripheral nerve regeneration. *Mol Neurobiol* 14(1–2):67–116

- Galea V, Fehlings D, Kirsch S, McComas A (2001) Depletion and sizes of motor units in spinal muscular atrophy. *Muscle Nerve* 24(9):1168–1172
- Gallagher TA, Simon NG, Kliot M (2015) Diffusion tensor imaging to visualize axons in the setting of nerve injury and recovery. *Neurosurg Focus* 39(3):E10
- Gaudet AD, Popovich PG, Ramer MS (2011) Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. *J Neuroinflammation* 8:110–110
- Geuna S, Raimondo S, Ronchi G, Di Scipio F, Tos P, Czaja K, Fornaro M (2009) Chapter 3: Histology of the peripheral nerve and changes occurring during nerve regeneration. *Int Rev Neurobiol* 87:27–46
- Giannini C, Lais AC, Dyck PJ (1989) Number, size, and class of peripheral nerve fibers regenerating after crush, multiple crush, and graft. *Brain Res* 500(1):131–138
- Gooch CL, Harati Y (2000) Motor unit number estimation, ALS and clinical trials. *Amyotroph Lateral Scler Other Motor Neuron Disorders* 1(2):71–82
- Gooch CL, Doherty TJ, Chan KM, Bromberg MB, Lewis RA, Stashuk DW, Berger MJ, Andary MT, Daube JR (2014) Motor unit number estimation: a technology and literature review. *Muscle Nerve* 50(6):884–893
- Gordon T, Chan KM, Sulaiman OAR, Udina E, Amirjani N, Brushart TM (2009) Accelerating axon growth to overcome limitations in functional recovery after peripheral nerve injury. *Neurosurgery* 65(4 Suppl):A132–A144
- Gydikov A, Gerilovsky L, Kostov K, Gatev P (1980) Influence of some features of the muscle structure on the potentials of motor units, recorded by means of different types of needle electrodes. *Electromyogr Clin Neurophysiol* 20(4-5):299–321
- Hansson T, Brismar T (2003) Loss of sensory discrimination after median nerve injury and activation in the primary somatosensory cortex on functional magnetic resonance imaging. *J Neurosurg* 99(1):100–105
- He B, Zhu Z, Zhu Q, Zhou X, Zheng C, Li P, Zhu S, Liu X, Zhu J (2014) Factors predicting sensory and motor recovery after the repair of upper limb peripheral nerve injuries. *Neural Regen Res* 9(6):661–672
- Heckel A, Weiler M, Xia A, Ruetters M, Pham M, Bendszus M, Heiland S, Baeumer P (2015) Peripheral nerve diffusion tensor imaging: assessment of axon and myelin sheath integrity. *PLoS One* 10(6):e0130833
- Hilz MJ, Axelrod FB, Hermann K, Haertl U, Duetsch M, Neundorfer B (1998) Normative values of vibratory perception in 530 children, juveniles and adults aged 3–79 years. *J Neurol Sci* 159(2):219–225
- Hoffman H (1950) Local re-innervation in partially denervated muscle: a histo-physiological study. *Aust J Exp Biol Med Sci* 28(4):383–398
- Holzgrefe RE, Wagner ER, Singer AD, Daly CA (2019) Imaging of the peripheral nerve: concepts and future direction of magnetic resonance neurography and ultrasound. *J Hand Surg* 44(12):1066–1079
- Howe FA, Filler AG, Bell BA, Griffiths JR (1992) Magnetic resonance neurography. *Magn Reson Med* 28(2):328–338
- Hsueh IP, Lee MM, Hsieh CL (2002) The Action Research Arm Test: is it necessary for patients being tested to sit at a standardized table? *Clin Rehabil* 16(4):382–388
- Hughes SM, Taylor JM, Tapscott SJ, Gurley CM, Carter WJ, Peterson CA (1993) Selective accumulation of MyoD and myogenin mRNAs in fast and slow adult skeletal muscle is controlled by innervation and hormones. *Development* 118(4):1137–1147
- Hundepool CA, Bulstra LF, Kotsougiani D, Friedrich PF, Hovius SER, Bishop AT, Shin AY (2018) Comparable functional motor outcomes after repair of peripheral nerve injury with an elastase-processed allograft in a rat sciatic nerve model. *Microsurgery* 38(7):772–779
- Ikeda M, Oka Y (2012) The relationship between nerve conduction velocity and fiber morphology during peripheral nerve regeneration. *Brain Behav* 2(4):382–390
- Jacobsen AB, Bostock H, Tankisi H (2018) CMAP scan MUNE (MScan) – a novel motor unit number estimation (MUNE) method. *J Vis Exp* 136:56805

- Jayakumar P, Overbeek CL, Lamb S, Williams M, Funes C, Gwilym S, Ring D, Vranceanu AM (2018) What factors are associated with disability after upper extremity injuries? A systematic review. *Clin Orthop Relat Res* 476(11):2190–2215
- Jeon T, Fung MM, Koch KM, Tan ET, Sneag DB (2018) Peripheral nerve diffusion tensor imaging: overview, pitfalls, and future directions. *J Magn Reson Imaging* 47(5):1171–1189
- Jerosch-Herold C (2005) Assessment of sensibility after nerve injury and repair: a systematic review of evidence for validity, reliability and responsiveness of tests. *J Hand Surg Br* 30(3):252–264
- Jespersen SN (2012) Equivalence of double and single wave vector diffusion contrast at low diffusion weighting. *NMR Biomed* 25(6):813–818
- Jessen KR, Arthur-Farraj P (2019) Repair Schwann cell update: adaptive reprogramming, EMT, and stemness in regenerating nerves. *Glia* 67(3):421–437
- Jessen KR, Mirsky R (2019) The success and failure of the schwann cell response to nerve injury. *Front Cell Neurosci* 13:33–33
- Jiang X, Li H, Xie J, Zhao P, Gore JC, Xu J (2016) Quantification of cell size using temporal diffusion spectroscopy. *Magn Reson Med* 75(3):1076–1085
- Joyce NC, Carter GT (2013) Electrodiagnosis in persons with amyotrophic lateral sclerosis. *PM R* 5(5 Suppl):S89–S95
- Kadrie HA, Yates SK, Milner-Brown HS, Brown WF (1976) Multiple point electrical stimulation of ulnar and median nerves. *J Neurol Neurosurg Psychiatry* 39(10):973–985
- Kakkar LS, Bennett OF, Siow B, Richardson S, Ianus A, Quick T, Atkinson D, Phillips JB, Drobnyak I (2018) Low frequency oscillating gradient spin-echo sequences improve sensitivity to axon diameter: an experimental study in viable nerve tissue. *Neuroimage* 182:314–328
- Kamath S, Venkatanarasimha N, Walsh MA, Hughes PM (2008) MRI appearance of muscle denervation. *Skeletal Radiol* 37(5):397–404
- Kaya Y, Sarikcioglu L (2015) Sir Herbert Seddon (1903–1977) and his classification scheme for peripheral nerve injury. *Childs Nerv Syst* 31(2):177–180
- Kikuchi Y, Nakamura T, Takayama S, Horiuchi Y, Toyama Y (2003) MR imaging in the diagnosis of denervated and reinnervated skeletal muscles: experimental study in rats. *Radiology* 229(3):861–867
- Kline DG (2012) Chapter 16 – Operative neurophysiology of the brachial plexus intraoperative electrodiagnostic studies. In: Chung KC, Yang LJS, McGillicuddy JE (eds) *Practical management of pediatric and adult brachial plexus palsies*. W.B. Saunders, Philadelphia, pp 212–219
- Kline DG, Happel LT (1993) Penfield Lecture. A quarter century's experience with intraoperative nerve action potential recording. *Can J Neurol Sci* 20(1):3–10
- Kobayashi J, Mackinnon SE, Watanabe O, Ball DJ, Gu XM, Hunter DA, Kuzon WM Jr (1997) The effect of duration of muscle denervation on functional recovery in the rat model. *Muscle Nerve* 20(7):858–866
- Koltzenburg M, Bendszus M (2004) Imaging of peripheral nerve lesions. *Curr Opin Neurol* 17(5):621–626
- Kostromina T, Dow D, Dennis R, Miller R, Faulkner JA (2005) Comparison of gene expression of 2-mo denervated, 2-mo stimulated-denervated, and control rat skeletal muscles. *Physiol Genom* 22:227–243
- Kraft GH (1990) Fibrillation potential amplitude and muscle atrophy following peripheral nerve injury. *Muscle Nerve* 13(9):814–821
- Krarup C, Boeckstyns M, Ibsen A, Moldovan M, Archibald S (2016) Remodeling of motor units after nerve regeneration studied by quantitative electromyography. *Clin Neurophysiol* 127(2):1675–1682
- Kullmer K, Sievers KW, Reimers CD, Rompe JD, Muller-Felber W, Nagele M, Harland U (1998) Changes of sonographic, magnetic resonance tomographic, electromyographic, and histopathologic findings within a 2-month period of examinations after experimental muscle denervation. *Arch Orthop Trauma Surg* 117(4-5):228–234

- Kuntz C t, Blake L, Britz G, Filler A, Hayes CE, Goodkin R, Tsuruda J, Maravilla K, Kliot M (1996) Magnetic resonance neurography of peripheral nerve lesions in the lower extremity. *Neurosurgery* 39(4):750–756. discussion 756–757
- Lawson VH, Gordon Smith A, Bromberg MB (2003) Assessment of axonal loss in Charcot-Marie-Tooth neuropathies. *Exp Neurol* 184(2):753–757
- Lee SK, Wolfe SW (2000) Peripheral nerve injury and repair. *J Am Acad Orthop Surg* 8(4):243–252
- Lee MB, David AH, Rajiv M, Erin W, Marco V (2004) Neurogenic motor evoked potentials: role in brachial plexus surgery. *Neurosurg Focus FOC* 16(5):607–610
- Li X, Chen J, Hong G, Sun C, Wu X, Peng MJ, Zeng G (2013) In vivo DTI longitudinal measurements of acute sciatic nerve traction injury and the association with pathological and functional changes. *Eur J Radiol* 82(11):e707–e714
- Li R, Liu Z, Pan Y, Chen L, Zhang Z, Lu L (2014) Peripheral nerve injuries treatment: a systematic review. *Cell Biochem Biophys* 68(3):449–454
- Lowitzsch K, Hopf HC, Galland J (1977) Changes of sensory conduction velocity and refractory periods with decreasing tissue temperature in man. *J Neurol* 216(3):181–188
- Lubinska L (1982) Patterns of Wallerian degeneration of myelinated fibres in short and long peripheral stumps and in isolated segments of rat phrenic nerve. Interpretation of the role of axoplasmic flow of the trophic factor. *Brain Res* 233(2):227–240
- Ma J, Shen J, Lee CA, Elsaidi GA, Smith TL, Walker FO, Rushing JT, Tan KH, Koman LA, Smith BP (2005) Gene expression of nAChR, SNAP-25 and GAP-43 in skeletal muscles following botulinum toxin A injection: a study in rats. *J Orthop Res* 23(2):302–309
- Ma J, Shen J, Garrett JP, Lee CA, Li Z, Elsaidi GA, Ritting A, Hick J, Tan KH, Smith TL, Smith BP, Koman LA (2007) Gene expression of myogenic regulatory factors, nicotinic acetylcholine receptor subunits, and GAP-43 in skeletal muscle following denervation in a rat model. *J Orthop Res* 25(11):1498–1505
- MacAvoy MC, Green DP (2007) Critical reappraisal of Medical Research Council muscle testing for elbow flexion. *J Hand Surg Am* 32(2):149–153
- Macdonald WM (1918) Tinel's sign in peripheral nerve lesions. *Br Med J* 2(3001):6–8
- Mackinnon, S. E. and A. L. Dellon (1988). *Surgery of the peripheral nerve*. New York; Stuttgart; New York, Thieme Medical Publishers ; G. Thieme Verlag.
- Mackinnon SE, Novak CB, Myckatyn TM, Tung TH (2005) Results of reinnervation of the biceps and brachialis muscles with a double fascicular transfer for elbow flexion. *J Hand Surg* 30(5):978–985
- Maglioni S, Giraldo DL, Duarte J, Velasco N, Romero E (2018) Description of brain volumetric changes in Alzheimer disease using region-based morphometry. SPIE, 14th International Symposium on Medical Information Processing and Analysis, Mazatlán, Mexico
- Mallik A, Weir AI (2005) Nerve conduction studies: essentials and pitfalls in practice. *J Neurol Neurosurg Psychiatry* 76(suppl 2):ii23–ii31
- Mansukhani KA (2013) Electrodiagnosis in traumatic brachial plexus injury. *Ann Indian Acad Neurol* 16(1):19–25
- Mar FM, Bonni A, Sousa MM (2014) Cell intrinsic control of axon regeneration. *EMBO Rep* 15(3):254–263
- Maravilla KR, Bowen BC (1998) Imaging of the peripheral nervous system: evaluation of peripheral neuropathy and plexopathy. *AJNR Am J Neuroradiol* 19(6):1011–1023
- McComas AJ, Fawcett PRW, Campbell MJ, Sica REP (1971) Electrophysiological estimation of the number of motor units within a human muscle. *J Neurol Neurosurg Psychiatry* 34(2):121
- McDonnell M (2008) Action research arm test. *Aust J Physiother* 54(3):220
- Mendler L, Pintér S, Kiricsi M, Baka Z, Dux L (2007) Regeneration of reinnervated rat soleus muscle is accompanied by fiber transition toward a faster phenotype. *J Histochem Cytochem* 56(2):111–123
- Menorca RMG, Fussell TS, Elfar JC (2013) Nerve physiology: mechanisms of injury and recovery. *Hand Clin* 29(3):317–330

- Mercredi M, Vincent TJ, Bidinosti CP, Martin M (2017) Assessing the accuracy of using oscillating gradient spin echo sequences with AxCaliber to infer micron-sized axon diameters. *Magma* 30 (1):1–14
- Miledi R, Slater CR (1970) On the degeneration of rat neuromuscular junctions after nerve section. *J Physiol* 207(2):507–528
- Millesi H (1985) Peripheral nerve repair: terminology, questions, and facts. *J Reconstr Microsurg* 2 (1):21–31
- Mitolo M, Stanzani-Maserati M, Capellari S, Testa C, Rucci P, Poda R, Oppi F, Gallassi R, Sambati L, Rizzo G, Parchi P, Evangelisti S, Talozzi L, Tonon C, Lodi R, Liguori R (2019) Predicting conversion from mild cognitive impairment to Alzheimer's disease using brain (1)H-MRS and volumetric changes: a two- year retrospective follow-up study. *Neuroimage Clin* 23:101843
- Morisaki S, Kawai Y, Umeda M, Nishi M, Oda R, Fujiwara H, Yamada K, Higuchi T, Tanaka C, Kawata M, Kubo T (2011) In vivo assessment of peripheral nerve regeneration by diffusion tensor imaging. *J Magn Reson Imaging* 33(3):535–542
- Moser T, Kremer S, Holl N (2009) Imagerie du nerf peripherique: anatomie, techniques d'exploration et principales pathologies. *J Radiol* 90(10):1448
- Murphy P, Koh DM (2010) Imaging in clinical trials. *Cancer Imaging* 10(1A):S74–S82
- Mydlarz WK, Boahene KO (2013) Sunderland classification of nerve injury. In: Kountakis SE (ed) *Encyclopedia of otolaryngology, head and neck surgery*. Springer, Berlin/Heidelberg, pp 2615–2616
- Nandedkar SD, Barkhaus PE (2007) Contribution of reference electrode to the compound muscle action potential. *Muscle Nerve* 36(1):87–92
- Nandedkar SD, Nandedkar DS, Barkhaus PE, Stalberg EV (2004) Motor unit number index (MUNIX). *IEEE Trans Biomed Eng* 51(12):2209–2211
- Navarro X (2016) Functional evaluation of peripheral nerve regeneration and target reinnervation in animal models: a critical overview. *Eur J Neurosci* 43(3):271–286
- Ohana M, Moser T, Moussaoui A, Kremer S, Carlier RY, Liverneaux P, Dietemann JL (2014) Current and future imaging of the peripheral nervous system. *Diagn Interv Imaging* 95(1):17–26
- Panegyres PK, Moore N, Gibson R, Rushworth G, Donaghy M (1993) Thoracic outlet syndromes and magnetic resonance imaging. *Brain* 116(4):823–841
- Parsons EC Jr, Does MD, Gore JC (2006) Temporal diffusion spectroscopy: theory and implementation in restricted systems using oscillating gradients. *Magn Reson Med* 55(1):75–84
- Pavlov S, Grosheva M, Streppel M, Guntinas-Lichius O, Irintchev A, Skouras E, Angelova S, Kuerten S, Sinis N, Dunlop S, Angelov D (2008) Manually-stimulated recovery of motor function after facial nerve injury requires intact sensory input. *Experimental neurology* 211:292–300
- Petrone PM, Casamitjana A, Falcon C, Artigues M, Operto G, Skouras S, Cacciaglia R, Molinuevo JL, Vilaplana V, Gispert JD, Salvadó G (2018) Characteristic brain volumetric changes in the AD preclinical signature. *Alzheimers Dement* 14(7):P1235
- Pette D, Vrbova G (1985) Neural control of phenotypic expression in mammalian muscle fibers. *Muscle Nerve* 8(8):676–689
- Polak JF, Jolesz FA, Adams DF (1988) Magnetic resonance imaging of skeletal muscle. Prolongation of T1 and T2 subsequent to denervation. *Invest Radiol* 23(5):365–369
- Power GA, Dalton BH, Behm DG, Doherty TJ, Vandervoort AA, Rice CL (2012) Motor unit survival in lifelong runners is muscle dependent. *Med Sci Sports Exerc* 44(7):1235–1242
- Quick TJ, Singh AK, Fox M, Sinisi M, MacQuillan A (2016) A quantitative assessment of the functional recovery of flexion of the elbow after nerve transfer in patients with a brachial plexus injury. *Bone Joint J* 98-B(11):1517–1520
- Rainey EE, Petrey LB, Reynolds M, Agtarap S, Warren AM (2014) Psychological factors predicting outcome after traumatic injury: the role of resilience. *Am J Surg* 208(4):517–523
- Rayner MLD, Brown HL, Wilcox M, Phillips JB, Quick TJ (2019) Quantifying regeneration in patients following peripheral nerve injury. *J Plast Reconstr Aesthet Surg* 73(2):201–208

- Robert MB, Peggy M, Jean MM, Frank PH (1995) Continuous intraoperative electromyographic recording during spinal surgery. *J Neurosurg* 82(3):401–405
- Ronchi G, Raimondo S (2017) Chronically denervated distal nerve stump inhibits peripheral nerve regeneration. *Neural Regen Res* 12(5):739–740
- Rose D, Kurtis IA, Cynthia TC, John WE, Philip RW (2010) Magnetic resonance neurography for the evaluation of peripheral nerve, brachial plexus, and nerve root disorders. *J Neurosurg* 112(2):362–371
- Rosen B, Lundborg G (2003) A new model instrument for outcome after nerve repair. *Hand Clin* 19(3):463–470
- Rotshenker S (2011) Wallerian degeneration: the innate-immune response to traumatic nerve injury. *J Neuroinflammation* 8:109–109
- Saadat S, Eslami V, Rahimi-Movaghar V (2011) The incidence of peripheral nerve injury in trauma patients in Iran. *Ulusal travma ve acil cerrahi dergisi = Turk J Trauma Emerg Surg* 17(6):539–544
- Saito H, Dahlin LB (2008) Expression of ATF3 and axonal outgrowth are impaired after delayed nerve repair. *BMC Neurosci* 9(1):88–88
- Scheib J, Hoke A (2013) Advances in peripheral nerve regeneration. *Nat Rev Neurol* 9(12):668–676
- Seddon HJB (1943) Three types of nerve injury. *Brain* 66(4):237–288
- Seddon HJ, Medawar PB, Smith H (1943) Rate of regeneration of peripheral nerves in man. *J Physiol* 102(2):191–215
- Seitz M, Grosheva M, Skouras E, Angelova SK, Ankerne J, Jungnickel J, Grothe C, Klimaschewski L, Hübbers C, Dunlop S, Angelov D (2011) Poor functional recovery and muscle poly-innervation after facial nerve injury in fibroblast growth factor-2^{-/-} mice can be improved by manual stimulation of denervated vibrissal muscles. *Neuroscience* 182:241–247
- Seror P (2017) Neuralgic amyotrophy. An update. *Joint Bone Spine* 84(2):153–158
- Severinsen K, Jakobsen J (2009) Chromatolysis. In: Binder MD, Hirokawa N, Windhorst U (eds) *Encyclopedia of neuroscience*. Springer, Berlin/Heidelberg, pp 714–716
- Shemesh N, Alvarez GA, Frydman L (2015) Size distribution imaging by non-uniform oscillating-gradient spin echo (NOGSE) MRI. *PLoS One* 10(7):e0133201
- Shen Q, Loewenstein DA, Potter E, Zhao W, Appel J, Greig MT, Raj A, Acevedo A, Schofield E, Barker W, Wu Y, Potter H, Duara R (2011) Volumetric and visual rating of magnetic resonance imaging scans in the diagnosis of amnesic mild cognitive impairment and Alzheimer's disease. *Alzheimers Dement* 7(4):e101–e108
- Simon NG, Talbott J, Chin CT, Kliot M (2016) Chapter 40 – Peripheral nerve imaging. In: Masdeu JC, González RG (eds) *Handbook of clinical neurology*, vol 136. Elsevier, Amsterdam, pp 811–826
- Slimp JC (2000) Intraoperative monitoring of nerve repairs. *Hand Clin* 16(1):25–36
- Smith S, Knight R (2011) Clinical neurophysiology in peripheral nerve injuries BT. In: Birch R (ed) *Surgical disorders of the peripheral nerves*. Springer, London, pp 191–229
- Smith JW, Thesleff S (1976) Spontaneous activity in denervated mouse diaphragm muscle. *J Physiol* 257(1):171–186
- Sobotka S, Mu L (2013) Force recovery and axonal regeneration of the sternomastoid muscle reinnervated with the end-to-end nerve anastomosis. *J Surg Res* 182(2):e51–e59
- Sollerman C, Ejekær A (1995) Sollerman hand function test. A standardised method and its use in tetraplegic patients. *Scand J Plast Reconstr Surg Hand Surg* 29(2):167–176
- Stalberg E, Falck B (1997) The role of electromyography in neurology. *Electroencephalogr Clin Neurophysiol* 103(6):579–598
- Stanisz GJ, Mitha R, Munro CA, Henkelman RM (2001) MR properties of rat sciatic nerve following trauma. *Magn Reson Med* 45(3):415–420
- Stassart RM, Möbius W, Nave K-A, Edgar JM (2018) The axon-myelin unit in development and degenerative disease. *Front Neurosci* 12:467
- Sulaiman W, Gordon T (2013) Neurobiology of peripheral nerve injury, regeneration, and functional recovery: from bench top research to bedside application. *Ochsner J* 13(1):100–108

- Sullivan R, Dailey T, Duncan K, Abel N, Borlongan CV (2016) Peripheral nerve injury: stem cell therapy and peripheral nerve transfer. *Int J Mol Sci* 17(12):2101
- Sunderland S (1951) A classification of peripheral nerve injuries producing loss of function. *Brain* 74(4):491–516
- Sutter M, Eggspuehler A, Muller A, Dvorak J (2007) Multimodal intraoperative monitoring: an overview and proposal of methodology based on 1,017 cases. *Eur Spine J* 2(Suppl 2):S153–S161
- Tagliafico A, Tagliafico G, Martinoli C (2010) Nerve density: a new parameter to evaluate peripheral nerve pathology on ultrasound. preliminary study. *Ultrasound Med Biol* 36(10):1588–1593
- Takehara I, Chu J, Schwartz I, Aye HH (2004) Motor unit action potential (MUAP) parameters affected by editing duration cursors. *Electromyogr Clin Neurophysiol* 44(5):265–269
- Timothy H (2014) Nerve injury: Classification, clinical assessment, investigation, and management. In: Handchirurgie Weltweit e.V (ed) Living textbook of hand surgery. GMS, Cologne
- Titelbaum DS, Frazier JL, Grossman RI, Joseph PM, Yu LT, Kassab EA, Hickey WF, LaRossa D, Brown MJ (1989) Wallerian degeneration and inflammation in rat peripheral nerve detected by in vivo MR imaging. *AJNR Am J Neuroradiol* 10(4):741–746
- Tung TH, Mackinnon SE (2010) Nerve transfers: indications, techniques, and outcomes. *J Hand Surg* 35(2):332–341
- Ultee J, Hundepool CA, Nijhuis TH, van Baar AL, Hovius SE (2013) Early posttraumatic psychological stress following peripheral nerve injury: a prospective study. *J Plast Reconstr Aesthet Surg* 66(10):1316–1321
- Valero-Cabr   A, Navarro X (2001) H reflex restitution and facilitation after different types of peripheral nerve injury and repair. *Brain Res* 919(2):302–312
- van Rosmalen M, Lieba-Samal D, Pillen S, van Alfen N (2019) Ultrasound of peripheral nerves in neuralgic amyotrophy. *Muscle Nerve* 59(1):55–59
- Viddeleer AR, Sijens PE, van Ooyen PMA, Kuypers PDL, Hovius SER, Oudkerk M (2012) Sequential MR imaging of denervated and reinnervated skeletal muscle as correlated to functional outcome. *Radiology* 264(2):522–530
- Visser LH (2006) High-resolution sonography of the common peroneal nerve: detection of intraneural ganglia. *Neurology* 67(8):1473
- Voytik SL, Przyborski M, Badylak SF, Konieczny SF (1993) Differential expression of muscle regulatory factor genes in normal and denervated adult rat hindlimb muscles. *Dev Dyn* 198(3):214–224
- Waller Augustus V, Owen R (1851) Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibres. Abstracts of the papers communicated to the Royal Society of London, vol 5, pp 924–925
- Wang FC, Delwaide PJ (1995) Number and relative size of thenar motor units estimated by an adapted multiple point stimulation method. *Muscle Nerve* 18(9):969–979
- Weinstein S (1993) Fifty years of somatosensory research: from the Semmes-Weinstein monofilaments to the Weinstein Enhanced Sensory Test. *J Hand Ther* 6(1):11–22; discussion 50
- Weis J, Kaussen M, Calvo S, Buonanno A (2000) Denervation induces a rapid nuclear accumulation of MRF4 in mature myofibers. *Dev Dyn* 218(3):438–451
- Weng LY, Hsieh CL, Tung KY, Wang TJ, Ou YC, Chen LR, Ban SL, Chen WW, Liu CF (2010) Excellent reliability of the Sollerman hand function test for patients with burned hands. *J Burn Care Res* 31(6):904–910
- Wessig C, Koltzenburg M, Reiners K, Solymosi L, Bendszus M (2004) Muscle magnetic resonance imaging of denervation and reinnervation: correlation with electrophysiology and histology. *Exp Neurol* 185(2):254–261
- Wilcox M, Gregory H, Powell R, Quick TJ, Phillips JB (2020) Strategies for peripheral nerve repair. *Curr Tissue Microenviron Rep* 1:49–59

- Willand MP, Chiang CD, Zhang JJ, Kemp SWP, Borschel GH, Gordon T (2015) Daily electrical muscle stimulation enhances functional recovery following nerve transection and repair in rats. *Neurorehabil Neural Repair* 29(7):690–700
- Windisch A, Gundersen K, Szabolcs MJ, Gruber H, Lomo T (1998) Fast to slow transformation of denervated and electrically stimulated rat muscle. *J Physiol* 510(2):623–632
- Wu P, Chawla A, Spinner RJ, Yu C, Yaszemski MJ, Windebank AJ, Wang H (2014) Key changes in denervated muscles and their impact on regeneration and reinnervation. *Neural Regen Res* 9 (20):1796–1809
- Yarnitsky D, Sprecher E (1994) Thermal testing: normative data and repeatability for various test algorithms. *J Neurol Sci* 125(1):39–45
- Zhao S, Kim DH, Kline DG, Beuerman RW, Thompson HW (1993) Somatosensory evoked potentials induced by stimulating a variable number of nerve fibers in rat. *Muscle Nerve* 16 (11):1220–1227
- Zivadinov R, Khan N, Medin J, Christoffersen P, Price J, Korn JR, Bonzani I, Dwyer MG, Bergsland N, Carl E, Silva D, Weinstock-Guttman B (2017) An observational study to assess brain MRI change and disease progression in multiple sclerosis clinical practice-the MS-MRIUS study. *J Neuroimaging* 27(3):339–347



Regenerative Therapies for Acquired Axonal Neuropathies

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Abstract

Peripheral neuropathies (PN) are the most common neurodegenerative disease globally. A variety of different insults lead to PN which include diabetes, cancer chemotherapy, and infectious diseases (such as HIV, *Campylobacter jejuni*, and hepatitis C). The clinical signs and symptoms depend on the extent of damage to motor, sensory, and autonomic fibers as well as the pain systems. Despite this heterogeneity of etiologies, the pathogenesis of PN is confined to one of several biological mechanisms that lead to eventual axonal degeneration. These molecular mechanisms allow identification of appropriate therapeutic targets to promote regeneration and functional recovery, but so far none of these preclinical discoveries resulted in clinical success. In addition, the poor ability of regenerating axons to reach their target represents a significant challenge to the development of effective regenerative therapies. This has stimulated research into new therapeutic delivery strategies. As a result, transdermal drug delivery and gene therapy have emerged as ideal candidates to circumvent these challenges. This chapter provides an insight into how these technologies can be harnessed to maximize the benefit of regenerative therapies for acquired axonal PN.

1 Introduction

Peripheral neuropathies (PN) represent a worldwide healthcare burden with an estimated prevalence of 2.4% among the global population (Osuntokun 1986; Martyn and Hughes 1997) and is associated with significant socioeconomic consequences (Jennum et al. 2015). The clinical manifestations, etiologies, and histories of PN are mixed. Systemic illnesses (such as diabetes mellitus), cancer chemotherapy, infectious diseases (such as HIV), autoimmune diseases, alcoholism, and environmental agents (such as organophosphates) are well-documented causes of PN (Cashman and Höke 2015; Landowski et al. 2016).

Clinical manifestations of PN are equally as diverse; pain, muscle weakness, altered sensation, atrophy, diminished coordination, and autonomic dysfunction or a combination of all are typical signs of PN. By extension, identifying the etiology of PN is often challenging.

Despite the variety in clinical manifestations and causes of PN, the mechanisms that cause axonal PN converge onto a select group of cellular and molecular mechanisms (Table 1) are metabolic dysregulation, covalent modifications (such as the modification of collagen and DNA by advanced glycation end products (AGEs) found in animal and patient models of diabetes mellitus (Brownlee et al. 1983; Tanaka et al. 1988; Mullokandov et al. 1994)), altered mitochondrial and endoplasmic reticulum function leading to excess production of reactive oxygen species,

Table 1 A summary of cellular and molecular mechanisms that underpin different peripheral neuropathies

Mechanism	Summary of pathogenesis	Clinical neuropathy
<i>Metabolic dysregulation</i>	• Hyperglycemia leads to dysregulation of the polyol, hexosamine, and pentose phosphate pathways (Gabbay et al. 1966; Yorek et al. 1987; Zhang et al. 2010) • Results in reactive intermediates that damage axons and Schwann cells (Gabbay et al. 1966; Yorek et al. 1987; Zhang et al. 2010)	• Diabetic neuropathy
<i>Covalent modifications</i>	• Formation of modified proteins; advanced glycation end products (AGEs) (Vlassara et al. 1981; Duran-Jimenez et al. 2009) • AGEs accumulate in Schwann cells and axons (Vlassara et al. 1981; Duran-Jimenez et al. 2009) • AGE products damage DNA and other biomolecules (Mullokandov et al. 1994) • DNA modification is also seen in chemotherapy-induced peripheral neurotoxicity (McDonald et al. 2005; Ta et al. 2006)	• Diabetic neuropathy • Chemotherapy-induced peripheral neurotoxicity
<i>Mitochondrial dysfunction</i>	• Mitochondria are thought to play an important role in neuropathy through mediating cell death and injury • Alterations in mitochondrial calcium signaling and DNA are thought to be at least partially responsible (Kidd et al. 2002; Mironov et al. 2005; Cashman and Höke 2015)	• Diabetic neuropathy • Chemotherapy-induced peripheral neurotoxicity • HIV neuropathy • Antiretroviral neuropathy
<i>Endoplasmic reticulum dysfunction</i>	• Experiments which reduced calcium concentration of endoplasmic reticulum found that cells manifested greater resistance to HIV envelope protein gp120 mediated cell death (Höke et al. 2009) • Similar responses have been reported upon exposure of Dorsal Root Ganglia to a number of antineoplastic agents (Acharjee et al. 2010)	• Chemotherapy-induced peripheral neurotoxicity • HIV neuropathy
<i>Reactive oxygen and nitrogen species</i>	• Mitochondria produce reactive species (hydrogen peroxide, hydroxide radicals and nitric oxide) (Barja and Herrero 1998) • These reactive species can damage proteins and DNA through covalent modification (Sima et al. 2000; Naudi et al. 2012)	• Diabetic neuropathy • Chemotherapy-induced peripheral neurotoxicity • HIV neuropathy
<i>Intracellular and inflammatory signaling</i>	• Receptors for AGEs have been shown to be pertinent in inducing oxidative stress and NF-κB induction (Yan et al. 1994) • Dysregulation of nitric oxide synthases are thought to lead to an increased concentration of reactive nitrogen intermediates leading to axonal and glial damage (Zochodne and Levy 2005)	• Diabetic neuropathy • HIV neuropathy • Hepatitis C neuropathy • Chemotherapy-induced peripheral neurotoxicity

(continued)

Table 1 (continued)

Mechanism	Summary of pathogenesis	Clinical neuropathy
	• Antiretrovirals increase inflammatory signaling (Zheng et al. 2011) and activation of apoptotic pathways (Bodner et al. 2004) • Inappropriate inflammatory signaling is thought to contribute to the onset of Hepatitis C neuropathy (Cashman and Höke 2015)	
<i>Slowed axonal transport</i>	• Impaired axonal transport of cytoskeletal components has been reported in animal models of diabetes (Medori et al. 1985) • A number of chemotherapeutic agents are thought to impair axonal transport by interfering with microtubule stabilization and/or covalent modification of tubulin (Theiss and Meller 2000; Gornstein and Schwarz 2014; Cashman and Höke 2015)	• Diabetic neuropathy • Chemotherapy-induced peripheral neurotoxicity
<i>Channelopathy</i>	• Abnormalities in Dorsal Root Ganglia and nerves following the administration of oxaliplatin can be reversed by the application of a sodium channel blocker (Adelsberger et al. 2000). This suggests involvement of sodium channels in chemotherapy induced peripheral neurotoxicity • Injection of the lipooligosaccharide of <i>C. jejuni</i> into rabbits has been shown to induce antibodies to GM1 and paralysis consistent with Guillian–Barré syndrome (Yuki et al. 2001)	• Chemotherapy-induced peripheral neurotoxicity • Guillian–Barré syndrome

altered intracellular and inflammatory signaling, and slowed axonal transport and channelopathy.

While therapies that modulate these pathological processes and promote regeneration have been well documented in animal models (Bota and Fodor 2019), clinical translation is awaited. This is largely attributable to a number of reasons. First, little is known about the final molecular mechanisms responsible for axonal degeneration itself. Improved characterization of these mechanisms would benefit the development of new therapeutics that prevent progressive axonal degeneration. This would create an environment which would maximize the benefit of regenerative therapies. Second, improved outcome measures that are sensitive and responsive to cellular and molecular mechanisms that underpin PN are awaited. Improved outcome measures would allow subclinical changes to be detected allowing earlier intervention before extensive axonal degeneration has ensued and facilitate the clinical translation of regenerative therapies for acquired axonal PN. Finally, new therapeutic delivery strategies are required in order to minimize off-target effects and maximize the effectiveness of any regenerative therapy. It is for these reasons that the mainstay treatment for PN remains largely focused on symptom control and/or preventing further degenerative changes.

PN can affect different components of the PNS; therefore, it is necessary to understand the conduction and myelination properties of axons as well as the anatomy of nerves that innervate the skin.

2 Innervation of the Skin

Sensory neurons of the dorsal root ganglia (DRG) are components of the nervous system most often affected in PNs. These neurons convey many different kinds of information from the periphery to the central nervous system (CNS). Therefore, these neurons represent a heterogeneous group in order to carry out their functions. Sensory neurons are commonly stratified according to the Erlanger–Gasser classification (Table 2).

Epidermal nerve fibers (ENFs) are nerves that innervate the most superficial layer of the skin: the epidermis. The majority of ENFs comprise of small C and Aδ fibers. The properties of these fibers are most susceptible to the pathological processes associated with small fiber neuropathies. The density and morphology of ENFs as determined from skin biopsy are universally deployed to diagnose PNs (Lubec et al. 1999; Lauria and Devigili 2007; Azhary et al. 2010).

Although there is some evidence that suggest increasing overlap in the functions of different groups of sensory neurons (Bargmann and Horvitz 1991; Julius and Basbaum 2001; Landowski et al. 2016), there is a general consensus that C-fibers are unmyelinated and have a role in nociception and heat detection (Julius and Basbaum 2001); A-δ fibers have a thin layer of myelin and convey information about mechanical stimuli; A-α and A-β represent heavily myelinated fibers allowing for fast conduction of proprioceptive information (Table 1).

Moving toward the CNS, more heavily myelinated fibers tend to enter the spinal cord more medially where they terminate at deeper lamina of the dorsal horn. This contrasts with smaller, unmyelinated fibers which terminate more laterally at more

Table 2 Sensory nerves and their properties

Erlanger–Gasser classification	Diameter (μm)	Conduction velocity (m/s)	Myelination status	Associated sensory receptor
Aα	13–20	80–120	Yes	Primary receptors of the muscle spindle
Aα	13–20	80–120	Yes	Golgi tendon organ
Aβ	6–12	33–75	Yes	Secondary receptors of the muscle spindle
Aδ	1–5	3–30	Thin	Cold thermoreceptors Free nerve endings of touch and pressure Nociceptors of lateral spinothalamic tract
C	0.5–2.0	0.5–2.0	No	Nociceptors of anterior spinothalamic tract Warmth receptors

superficial lamina of the dorsal horn. Deep to the basement membrane of the epidermis, ENFs are ensheathed by Schwann cells. As they traverse more distally toward the basement membrane of the epidermis, they lose their Schwann cell ensheathment and continue from the dermis to innervate the epidermis vertically where they form multiple fine terminal branches.

The degree of ENF loss does not always correlate with the severity of the PN (Lauria and Devigili 2007). However, considering the loss of ENFs in the skin along with axonal swelling demonstrates closer correlations with severity of the PN in the feet in a number of pathologies; DN, chemotherapy agent paclitaxel, idiopathic neuropathy and AIDS (Lauria and Devigili 2007). A number of clinical manifestations have been reported and depend to some extent on the insult.

3 Clinical Overview of Acquired Axonal Neuropathies

3.1 Diabetes

Diabetes represents the fastest growing chronic disease with a global prevalence of 9% in those over 18 (WHO 2011). It is thought that around 50% of those with a diagnosis of diabetes suffer from diabetic neuropathy (Ziegler et al. 1992; Dyck et al. 1993; Won et al. 2014). A number of different types of DN have been reported but by far the most common is a distal symmetrical sensory or sensorimotor polyneuropathy often accompanied by an autonomic neuropathy (Palumbo et al. 1978; Sinnreich et al. 2005).

Both subtypes of PN are length dependent and progress along a distal-to-proximal gradient (Palumbo et al. 1978; Sinnreich et al. 2005). This often manifests in the classical clinical presentation of a “glove and stocking” distribution of symptoms (Palumbo et al. 1978; Sinnreich et al. 2005). A difference between DN and other neuropathies such as CIPN is that onset is often insidious (Clements Jr and Bell 1982). Patients may describe symptoms that suggest neuropathic pain, allodynia, and/or numbness (Dyck et al. 1993; IDF 2014). Symptoms can result in a number of functional morbidities that range widely from moderate to severe due to an increased frequency of falls, ulceration, and fractures (Dyck et al. 1993).

3.2 Chemotherapy-Induced Peripheral Neurotoxicity

The development of new anti-neoplastic agents has improved outcomes for cancer patients significantly (Newlands 1978; Wright 1984). However, many of these agents are associated with long lasting toxic effects (Lema et al. 2010). As remission rates have increased dramatically over recent decades, the incidence of chemotherapy-induced neurotoxicity (CIPN) has seen a significant increase (Argyriou et al. 2014; Cavaletti 2014). It is thought that anywhere between 30% and 80% of patients treated with an antineoplastic agent will go on to develop a peripheral neuropathy (Ferrier et al. 2013). The time to onset of CIPN is heterogeneous and can manifest in

acute and chronic forms; for example, cisplatin predominantly leads to chronic neurological effects (Argyriou et al. 2014) while oxaliplatin demonstrates toxic neurological effects in both the acute and chronic settings (Argyriou et al. 2014). This suggests different mechanisms are likely to be responsible for early and late pathology. Impaired motor function, pain, and numbness are among the most commonly reported symptoms by patients (Toftshagen 2010). In many cases, the severity of symptoms limits the dose of anti-neoplastic agent that can be prescribed leading to suboptimal treatment of the cancer (Toftshagen 2010). This diverse set of symptoms suggests that chemotherapeutic agents damage different types of axons (Han and Smith 2013). Moreover, some chemotherapeutic agents may damage some parts of the axons more than others. Specifically, it has been shown that certain agents preferentially affect the axon (axonopathy) while others damage the ganglion (ganglionopathy) (Han and Smith 2013).

Taxanes (paclitaxel), platinum containing compounds (cisplatin and oxaliplatin), and proteasome inhibitors (such as bortezomib) are by far the most common causes of CIPN (Argyriou et al. 2014). Patients often develop a length-dependent sensory neuropathy which is evidenced by pathological specimens manifesting axonal degeneration and secondary demyelination in a length-dependent manner (Sahenk et al. 1994). Vinca alkaloids are another group of antineoplastic agents that often cause a sensory and motor peripheral neuropathy. They include vinorelbine, vinblastine, and vincristine and have a similar mechanism of action to the Taxanes; they disrupt the microtubules that form the spindle apparatus and interfere with the M phase of the cell division. This leads to a number of central and peripheral effects which manifest as hypersensitivity and allodynia (Yang et al. 2014).

3.2.1 Neuropathy Secondary to Human Immunodeficiency Virus

Almost 40 million globally are thought to be infected with human immunodeficiency virus (HIV) (HIV/AIDS 2010). The nervous system is affected by the virus; patients often present with a neuropathy (McArthur et al. 2005; Smyth et al. 2007). The prevalence of PN among HIV patients is attributable to the virus itself and the drugs used to treat HIV (McArthur et al. 2005; Smyth et al. 2007). Clinically, HIV neuropathy presents as a predominantly sensory neuropathy with pain, absent ankle reflexes, and paraesthesias among the typical signs reported (Cornblath and McArthur 1988).

HIV is not thought to directly affect axons or Schwann cells. Although some studies have found HIV in nerve samples, the concentrations are very low (de La Monte et al. 1988; Chaunu et al. 1989). Together, this suggests that the virus most likely invades nerves through other cellular vehicles such as macrophages and T-cells (Jones et al. 2005). This theory is supported by studies that have demonstrated the presence of macrophage and T-cell particles in biopsied nerve samples (Jones et al. 2005). Moreover, HIV proteins have demonstrated co-localization with macrophages *ex vivo* (Hahn et al. 2008) suggesting the immune system plays an important role in the delivery of proteins to the nerve. Protein R (VPR) and gp120 are among the viral proteins that are thought to be the most pertinent in the development of neurotoxicity (Keswani et al. 2002; Kamerman et al. 2012).

Therefore, the principal mechanisms that underpin HIV neuropathy are likely to be attributable to be damage to the nerve from viral proteins carried by immune cells.

3.2.2 Toxicity of Antiretroviral Drugs

Axonal damage caused by HIV is often compounded by toxic effects of the antiretroviral drugs used for treatment. Antiretrovirals have been shown to lead to aberrant mitochondrial function by inhibiting replication of the mitochondrial genome (Keilbaugh et al. 1997; Cashman and Höke 2015). As a result, a number of components of the electron transport chain cannot be manufactured leading to uncoupled transport and thus reduced oxidative phosphorylation (Keilbaugh et al. 1997). This may lead to an increased demand on the glycolysis pathway and therefore increased production of lactic acid. This is hypothesized is supported by findings which have shown that patients on nucleoside analogues demonstrate hyperlactemia which resolves on cessation of the drug (Boubaker et al. 2001; Antoniou et al. 2003). It is also thought that a number of antiretrovirals increase inflammatory signaling leading to aberrant production of reactive intermediates and activation of apoptotic machinery (Bodner et al. 2004; Zheng et al. 2011).

3.2.3 Hepatitis-C-Virus-Associated Neuropathy

HCV is an RNA virus that is known to lead to a refractory, chronic infection. Liver cirrhosis and hepatocellular carcinoma may also ensue with chronic HCV infection. HCV is thought to have a prevalence among the general population of 2.35% (5 times more common than HIV) (Lavanchy 2011). The association between HCV and the onset of neurological symptoms such as encephalopathy and PN has been well documented. Among patients infected with HCV, 9% and 10% are thought to develop sensory and motor neuropathies respectively (Cacoub et al. 2000). A number of subtypes of HCV-induced neuropathy have been reported. Four subtypes have been well characterized in the literature based on the distribution of symptoms: polyneuropathy, mononeuritis multiplex, cranial neuropathy, and a combination of cranial neuropathy and polyneuropathy (Nemni et al. 2003). It is known that HCV does not directly infect muscles or nerves (Authier et al. 2003). This suggests that HCV exerts its neurotoxic effects indirectly through inflammatory mechanisms.

The onset of HCV-induced neuropathy has been linked with cryoglobulinemia (Cacoub et al. 2000; Cashman and Höke 2015). Cryoglobulins are immunoglobulins that aggregate at temperatures below 37 °C. Raised concentrations of cryoglobulin in the blood have been associated with a number of autoimmune diseases (Ferri et al. 2002; Cashman and Höke 2015). More recently, it has been shown that between 30% and 78% of patients with HCV found to have cryoglobulinemia (Nemni et al. 2003; Santoro et al. 2006). Higher levels of cryoglobulinemia are not well correlated with the severity of neuropathy (Nemni et al. 2003). However, there is evidence suggesting that cryoglobulinemia is predictive of a more diffuse neuropathy (Nemni et al. 2003). This clinical observation is important since it is well documented that cryoglobulinemia causes a systemic vasculitis (Authier et al. 2003). This suggests that the activation of inflammatory pathways and ischemic

injury plays an important role in the development and progression of HCV-induced peripheral neuropathy.

3.2.4 *Campylobacter-jejuni*-Associated Neuropathy

C. jejuni is one of the most common causes of gastroenteritis across the Western hemisphere (Fujimoto and Amako 1990). Post-infectious neuropathy typically occurs a week following infection and has an incidence of 1 in 1000 infections (Allos 2001). First described by Guillain, Barré, and Strohl (Guillain 1916), this neuropathy clinically presents as an acute, ascending, and predominantly motor neuropathy (Winer 2014). While Guillain–Barré syndrome (GBS) is rare, the complications can be life threatening; diaphragmatic and respiratory muscle paralysis may ensue (Winer 2014). A number of viruses have been associated with the onset of GBS although *C. jejuni* is thought to account for around 30% of all GBS cases (Allos 2001). Clinical reports have also demonstrated that a number of different subtypes of GBS exist (Winer 2014). Differentiation between these clinical syndromes is often made by the speed of symptom progression and distribution of functional impairment.

It is clear that there are many different etiologies that may lead to PN. Moreover, there are a diverse range of clinical manifestations of PN. Together, this suggests that PN is a heterogeneous pathology. However, the pathogenesis of PN converges onto one or more of the following six mechanisms: metabolic dysregulation, covalent modifications, altered mitochondrial and endoplasmic reticulum function leading to excess production of reactive oxygen species, altered intracellular and inflammatory signaling, and slowed axonal transport and channelopathy (Cashman and Höke 2015). An understanding of the cellular and molecular features that underpin these processes is necessary in order to develop therapies that can stop neural degeneration and promote regeneration.

4 New Insights into Cellular and Molecular Mechanisms of Axonal Degeneration

Despite the heterogeneity of etiologies, the pathogenesis of PN is confined to one or more of the following six biological mechanisms that lead to eventual axonal degeneration: metabolic dysregulation, covalent modifications, altered mitochondrial and endoplasmic reticulum function leading to excess production of reactive oxygen species, altered intracellular and inflammatory signaling, and impaired axonal transport and channel dysfunction (reviewed in (Cashman and Höke 2015). The end result of these various cellular and molecular pathways is, often length-dependent, distal-to-proximal degeneration of axons which may share many characteristics with Wallerian degeneration.

Following insult, nerves degenerate and debris is cleared through an active process known as Wallerian degeneration. This event is critical to allow successful nerve repair and regeneration (Rotshenker 2011). This pathway is comparable to apoptosis. However, it has been shown that Wallerian degeneration is not dependent

on the activity of caspases and apoptotic signaling (Finn et al. 2000). This suggests that Wallerian degeneration is mediated by a different set of molecular signals.

Over recent years, there have been two primary areas of advancement in the understanding of Wallerian degeneration. The first was the discovery of a spontaneous mutation in mice which resulted in slowed Wallerian degeneration when axons were crushed or transected (Lunn et al. 1989). Nerve transfer experiments subsequently appeared to suggest that this effect was due to an intrinsic property of axons (Glass et al. 1993). Further studies that blocked apoptotic pathway in the neuronal cell body confirmed also seemed to point toward a distinct mechanism (Deckwerth and Johnson 1994). This observation (termed Wallerian degeneration slow (*Wlds*)) was later attributed to a mutation. This mutation arose due to a fusion protein that was composed of the first 70 amino acids of a ubiquitination factor (Ube4B) and an enzyme that is implicated in the synthesis of NAD⁺ (Mack et al. 2001). It is well documented that NAD⁺ and its isoforms have a pertinent role in mediating axon preservation (Coleman and Freeman 2010). Moreover, it has been shown that this *Wlds* phenotype is resistant to neuropathy induced by paclitaxel (Wang et al. 2002). Together this suggests there is a shared pathway of axon degeneration between Wallerian degeneration and paclitaxel.

The second was the identification of a molecule known as *Sarm1*. Genetic screening of *Drosophila* demonstrated that the deletion of *dSarm/Sarm1* delayed the onset of Wallerian degeneration (Osterloh et al. 2012). The role of *Sarm1* in the delay of Wallerian degeneration was further confirmed by RNAi studies (Gerdts et al. 2013). It is thought that *Sarm1* promotes axonal degeneration by neutralizing NAD⁺ (Gerdts et al. 2015). Moreover, a previous study demonstrated that *Sarm1* expression is necessary for cell death and axonal degeneration induced by depolarization of the mitochondrial membrane (Summers et al. 2014). Recent studies have confirmed the role of *Sarm1* in mediating axon degeneration caused by both toxic and metabolic insults (Geisler et al. 2016, 2019; Turkiew et al. 2017; Cheng et al. 2019).

This understanding of the fundamental cellular and molecular mechanisms that underpin PNs has been used to develop a number of therapies to promote regeneration and recovery. However, there are a number of challenges to clinical translation. Sensitive and responsive outcome measures are needed in order to trial new therapies to promote regeneration and functional recovery.

5 Outcome Measures to Evaluate Regeneration in Acquired Axonal Neuropathies

Developing appropriate tools to evaluate regeneration of axons in a timely manner in both preclinical and clinical models is paramount to the development of new therapeutics for axonal neuropathies. In preclinical studies, usually this is done by examining electrophysiological, behavioral, and histopathological outcome measures that can be administered over multiple time points in and in different animals. Unfortunately, many of these outcome measurement tools are

not easy to translate to clinical trials in humans. Although behavioral and electrophysiological outcome measures can be done in a repeated manner in a given patient, they suffer from the fact that behavioral (recovery of sensory or motor functions) or electrophysiological (emergence of evoked sensory or motor responses) readouts are only helpful once the axons regenerate enough to reach target tissues. They do not assess regeneration of axons “while” they are regenerating. Given the fact that human nerves regenerate so slowly (about an inch a month) (Sunderland 1947), these outcome measure require long-term clinical studies where the patient is exposed to experimental therapies without knowing if the drug is actually doing anything.

Currently, the primary end point that is accepted by the regulatory agencies is patient reported outcome measures such as various symptom-based composite scales (Merkies et al. 2012, 2015). These can be supplemented by structured neurological examinations and electrophysiology to give a more comprehensive composite score of the state of the peripheral nerves (Cornblath et al. 1999), but these add extra variability and are cumbersome for multicenter clinical trials. Because of that, there has been a move to simplify such comprehensive composite scales in evaluation of peripheral nerves and validate new outcome measures against the old ones in various clinical settings (Binda et al. 2015; Merkies et al. 2015).

A major issue with these outcome measures is that they are primarily developed to evaluate symptoms and signs of peripheral neuropathies and not necessarily regeneration of peripheral axons. Potentially new imaging techniques can offer an outcome measure that is repeatable and monitor nerve regeneration as it happens. Although there have been some advances in preclinical models (Takagi et al. 2009; Lehmann et al. 2010; Yamasaki et al. 2015; Andersson et al. 2018), clinical translation has been lacking. Furthermore, the new MRI-based imaging techniques are more suitable for monitoring regeneration after traumatic injury where the axons regenerate in relative unison but may not be sensitive enough to monitor asynchronous regeneration in axonal peripheral neuropathies.

What is needed in the field is a way to monitor axons as they regenerate. This is accomplished in preclinical models through histopathological evaluations of regenerating axons at different locations along the nerve and at different times, which is not feasible in humans. However, over the past decades, two experimental models of human nerve regeneration have been developed (Rajan et al. 2003; Polydefkis et al. 2004) and used in clinical trials (Polydefkis et al. 2006; Hahn et al. 2007). In one of these models, topical application of capsaicin leads to degeneration of epidermal sensory fibers and repeated skin biopsies in the denervated area over several months allow one to measure rate and extent of reinnervation (Polydefkis et al. 2004). In the other model, a circular excisional biopsy is followed by a larger biopsy to evaluate regeneration of axotomized dermal and epidermal nerve fibers (Rajan et al. 2003). Both of these experimental models in humans allow one to test experimental drug's effect on nerve regeneration in a timely manner. However, it still needs to be seen if any drug that shows efficacy in one of these experimental models results in better clinical outcomes in a clinical target disease such as diabetic neuropathy or traumatic nerve injury.

6 Therapeutic Candidates for Regeneration in Acquired Axonal Neuropathies

The reasons for the declining ability of nerves to regenerate and recover from neuropathy has been at the forefront of research for as long as the function of nerves has been understood.

In 1830 that Theodor Schwann first reported the phenomenon of nerve regeneration in rabbits. A century later, Ramon y Cajal suggested that nerve regeneration was dependent upon a number of trophic signals and cues. The first substance found to promote nerve regeneration was stumbled upon when researchers identified a substance in a mouse sarcoma which promoted nerve growth called Nerve Growth Factor (NGF) (Cohen et al. 1954). A number of additional growth factors that promote neuroprotection and regeneration have since been identified (Terenghi 1999).

The majority of these drugs and factors act on a highly specialized sensori-motile unit known as a growth cone (Caudy and Bentley 1986). Located at the terminal end of a regenerating neurite, the growth cone is thought to be largely responsible for driving forward regenerating and developing axons (Sperry 1963; Hur et al. 2012). At the early stages of development, growth cones are sensitive and responsive to substrate-bound and soluble chemotactic cues spatially and temporally (Sperry 1963; Caudy and Bentley 1986). This allows axons to navigate the nerve gap and re-establish functional connections with their targets.

A number of therapeutic agents are thought to act on this structure in order to accelerate nerve regeneration and improve functional recovery (see chapter ► “Drug Therapies for Peripheral Nerve Injuries”) from neuropathies.

6.1 Growth Factors

Nerve growth factor is principally involved in the proliferation and maintenance of neurons. NGF is a ligand of the Trk receptor which leads to the activation of Erk1/2 and Akt molecules (Du et al. 2011). This promotes neurite outgrowth and accelerates neural regeneration in vitro and in vivo (Du et al. 2011). The Trk receptor has also been implicated in a number of pain syndromes (Lankford et al. 2013). The ability of NGF to modulate the regenerative capacity of axons and nociception is thought to be attributable to its ability to promote microtubule organization (Ketschek et al. 2015). NGF achieves this through increasing the expression of microtubule associated proteins (Ketschek et al. 2015). For this reason, NGF has been extensively explored as a potential regenerative therapy for a number of axonal neuropathies with mixed results (Apfel 2002). Other neurotrophic factors such as BDNF, GDNF, NT-3, and CNTF have demonstrated similar results promoting axonal regeneration in vitro and in vivo (see chapter ► “Drug Therapies for Peripheral Nerve Injuries”) (Ebadi et al. 1997; Apfel 2002; Anand 2004; Leininger et al. 2004; Calcutt et al. 2008).

Granulocyte-colony stimulating factor (G-CSF) is a member of the cytokine growth factor family. G-CSF stimulates the development and differentiation of

hematopoietic progenitor cells into neutrophils. In addition, G-CSF has been shown to modulate the effects and distribution of neutrophils (Pan et al. 2009). The administration of G-CSF in rodent models of diabetes has shown to protect against morphological changes associated with the onset and progression of diabetic neuropathy (Lee et al. 2013). While the molecular basis of this observation remains unclear, it is thought to be attributable to the actions of bone marrow-derived cells (Lee et al. 2013). Randomized controlled clinical trials that investigate the efficacy of G-CSF as a regenerative therapy for acquired axonal neuropathies are awaited.

Insulin-like growth factor 1 (IGF-1) is a pluripotent growth factor. IGF-1 has a number of functions in the CNS and the PNS (Rauskolb et al. 2017). IGF-1 supports neuronal survival and outgrowth (Rauskolb et al. 2017). Studies have shown that diabetic patients have reduced level of IGF-1 compared to healthy individuals (Rauskolb et al. 2017). Similarly, in animal models of diabetes, reduced IGF-1 levels have been reported in nerves at an early stage of disease (Migdalís et al. 1995; Guo et al. 1999). Moreover, transgenic mice deficient of IGF-1 develop diabetic polyneuropathy (Gao et al. 1999). Introduction of recombinant IGF-1 restores sensory and nerve conduction velocities to that of healthy controls (Gao et al. 1999). In addition, the administration of IGF-1 in animal models of nerve injury demonstrated improved functional recovery compared to controls (Rauskolb et al. 2017). The cellular and molecular mechanisms through which IGF-1 achieves these effects are far from understood. This will be key to the clinical translation of IGF-1 as a regenerative therapy for acquired axonal neuropathies.

A number of challenges arise when using growth factor as regenerative therapies. These challenges are characterized in strategies to deliver regenerative therapies for acquired axonal neuropathies.

6.2 Immunosuppressants

The activation of inflammatory pathways and autoimmune pathways has already been identified as a major contributor to the onset and progression of a number of neuropathies. By extension, many immunosuppressive therapies have been trialed in order to disrupt the pathological processes associated with a number of axonal neuropathies (Léger et al. 2016). Corticosteroids, methotrexate, immunoglobulins, plasma exchange, and IFN- β 1a have been deployed in different combinations, doses, and time points in neuropathies such as Guillian–Barré and HIV neuropathies (Léger et al. 2016).

A handful of these immunosuppressants demonstrate accelerated axonal regeneration and improved functional recovery in animal models of peripheral nerve injury (Tacrolimus, IFN- β and Glatiramer acetate are among those that have been explored) (Zanon et al. 2010; Luria et al. 2013; Bota and Fodor 2019). However, their application as regenerative therapies in acquired axonal neuropathies is awaited. This is largely attributable to differences in disease phenotype between neuropathies. A greater understanding of the cellular and molecular features that underpin pathological intracellular and inflammatory signaling is needed.

6.3 Hormones

Erythropoietin (EPO) is a renal cytokine that closely regulates hematopoiesis. EPO is also produced by a number of cells in the nervous system and is a ligand to the EPO receptor. This receptor has been detected on glial cells in both the CNS and PNS (Bota and Fodor 2019). Its activation has been associated with neuroprotection and improved recovery in animal models of nerve injury (Yin et al. 2010; Bota and Fodor 2019). In addition, EPO appears to have an antioxidant and anti-inflammatory effect (Yin et al. 2010). This has been shown to be effective in preventing the onset and progression of neuropathy in animal models of type II diabetes (Suarez-Mendez et al. 2018). Further work is needed to identify the molecular mechanism through which EPO mediates these effects.

Melatonin is a hormone produced by the pineal gland and is responsible for mediating the circadian rhythm. In addition, melatonin is secreted by a number of other tissues including the retina, bone marrow, skin, and gastrointestinal tract. This hormone is thought to play a number of important roles in multiple physiological processes including immunogenesis, tumor development, and mental status (Tordjman et al. 2017). Melatonin has demonstrated a significant antioxidant effect that is independent of receptor binding; it has been shown to directly neutralize reactive oxygen species (Tordjman et al. 2017). This is likely to ameliorate the oxidative damage that is a key mechanism in the development and progression of a number of axonal neuropathies. The administration of melatonin has been shown to promote neural regeneration and improve signs of neuropathy in animal models of taxane-induced neuropathy (Nahleh et al. 2010).

Acetyl-L-carnitine (ALC) is a mitochondrial peptide involved in the lipid transport. It is currently deployed by some clinicians in the treatment of neuropathic pain. ALC is thought to mediate its effect by reducing mitochondrial oxidative stress (Lee et al. 2014; Sun et al. 2017). In addition, ALC is thought to increase the sensitivity of the growth cone to NGF and increases the expression of proregenerative proteins such as Artemin and GDNF (Hershman et al. 2013; Chan et al. 2014). At a molecular level, ALC is thought to mediate these effects through activation of tyrosine kinase A and ERK-1/2 (Hershman et al. 2013; Chan et al. 2014). ALC has also been shown to decrease intracellular concentrations of apoptotic proteins such as caspase-3 and increasing the bioavailability of acetyl-CoA in the mitochondria (Hershman et al. 2013; Bota and Fodor 2019). Together, this is thought to antagonize the biological processes necessary for the progression of neuropathies. This provides some explanation for its efficacy in the treatment of taxane- and diabetic-related neuropathies (Di Stefano et al. 2019).

Oxytocin is a hormone released by the posterior pituitary gland. Physiologically, this hormone has a pertinent role in sexual production across both sexes. Recent studies have shown that Oxytocin may positively modulate the regenerative capacity of axons by activating NGF and IGF-1 (Gümüs et al. 2015). Animal models of type II diabetes have demonstrated that Oxytocin has a neuroprotective effect and even some mild recovery was reported (Erbaş et al. 2017). Therefore, further exploration of Oxytocin as a regenerative therapy for the treatment of axonal neuropathies is warranted.

Testosterone propionate is an androgen and anabolic steroid that is commonly deployed to replace the hormone testosterone. Administration of this hormone has been shown to increase BDNF expression and genes associated with the neural repair program such as GAP-43 (Sharma et al. 2010; Brown et al. 2013). Together, this promotes an increase in the extracellular protein neuritin leading to an increase in neurite outgrowth which has been demonstrated *in vitro* and *in vivo* (Sharma et al. 2010; Brown et al. 2013). Recent isolated case reports suggest that testosterone propionate may have some therapeutic effect in the treatment of autoimmune neuropathy (Dickerman et al. 1997). Randomized clinical trials that investigate the efficacy of testosterone propionate as a regenerative therapy are awaited.

Finally, exendin-4 is a long-lasting analogue of glucagon-like peptide-1 (GLP-1). GLP-1 is a 30-amino-acid-long peptide that is derived from post-translational modification of the proglucagon peptide. GLP-1 agonists increase insulin levels and reduce liver gluconeogenesis. Exendin-4 has been shown to mitigate the severity of diabetic neuropathy in animal models (Himeno et al. 2011; Kan et al. 2012). *In vitro* evidence suggests that Exendin-4 exerts these effects through direct actions on axons and Schwann cells themselves (Himeno et al. 2011; Kan et al. 2012). In addition, administration of Exendin-4 in animal models of nerve injury improved functional recovery compared to controls (Yamamoto et al. 2013).

6.4 Other Drugs

Metformin increases tissue sensitivity to insulin and decreases liver gluconeogenesis. As well as having antidiabetic properties, metformin has been shown to have antioxidant, anti-inflammatory, and neuroprotective effects (Esteghamati et al. 2013). For these reasons, metformin is thought to mitigate metabolic dysregulation and damage caused by reactive oxygen species which underpins the progression of a number of neuropathies. This is supported by recent clinical trials which investigated the use of metformin as a drug to protect colorectal cancer patients against oxaliplatin-induced chronic peripheral sensory neuropathy; patients who received metformin demonstrated significantly improved pain scores compared to controls (Beijers et al. 2014; El-Fatraty et al. 2018). In addition, metformin has been shown to accelerate neural regeneration in animal models of peripheral nerve injury (Ma et al. 2016a). Further work is necessary in order to establish which molecular pathways metformin acts on to lead to these effects.

Sildenafil is a selective phosphodiesterase-5 inhibitor and leads to the intracellular accumulation of c-GMP. In animal models, Sildenafil has been shown to promote angiogenesis, synaptogenesis as well as neural outgrowth (Pyriochou et al. 2007; Xiong et al. 2010). These effects are thought to be at least partially responsible for the improved axonal regeneration and functional recovery reported in animal models of peripheral nerve injury (Fang et al. 2013). This effect appears to extend to neuropathies; the administration of Sildenafil has been shown to ameliorate chronic neuropathy in type II diabetic mice (Wang et al. 2015a). Clinical reports also provide further support for the role of Sildenafil in the treatment of diabetic neuropathy;

patients who took Viagra demonstrated fewer clinical manifestations of neuropathy (Escobar-Jimenez 2002), but this observation awaits confirmation in a randomized trial.

Nimodipine is an antagonist of the voltage gated calcium channel. The importance of impaired vascular supply to the nerve in the progression of neuropathy has already been discussed. Nimodipine is thought to ameliorate this effect by preventing vasoconstriction. Improvements in nerve conduction velocity across a number of axonal neuropathies treated with Nimodipine have been well documented (Navia et al. 1998; Escobar-Jimenez 2002; Gupta et al. 2003; Wang et al. 2015a). In addition, Nimodipine has been shown to lead to calcium dependent depolarization of the growth cone (Fang et al. 2013). This effect is thought to be responsible for accelerated neural regeneration and improved functional outcomes in animal models of nerve injury treated with Nimodipine (Fang et al. 2013).

N-acetylcysteine (NAC) is a mucolytic and antioxidant agent. In addition, NAC has been shown to accelerate neural regeneration (Karlidag et al. 2012) although the cellular and molecular features which underpin this effect remain poorly understood. As a result, NAC has gained interest as a potential regenerative therapy for acquired axonopathies. Animal models of type II diabetes demonstrated that NAC protected against the progression of diabetic neuropathy (Tardiolo et al. 2018). More recently, early results from clinical trials are suggesting that NAC may protect against chemotherapy-induced neuropathies (Tardiolo et al. 2018), but a larger randomized trial is needed to validate such findings.

6.5 Vitamins

Methylcobalamin is an analogue of Vitamin B₁₂, which is imperative for axonal myelination. As a result, it has been explored as a potential therapy for a number of PN, particularly diabetic neuropathy (Jayabalan and Low 2016). In addition, there is some evidence suggesting that Methylcobalamin may be an effective regenerative therapy; it has been shown that Methylcobalamin activates the protein kinases Erk 1/2 and Ark which promotes neurite outgrowth and branching (Okada et al. 2010; Bota and Fodor 2019). This is correlated with functional and histological improvements in animal models of nerve injury (Okada et al. 2010; Bota and Fodor 2019). A meta-analysis of clinical trials suggests that Methylcobalamin may improve symptoms of diabetic neuropathy more than electrophysiological measures of nerve function (Xu et al. 2013).

All-trans retinoic acid (ATRA) is a derivative of Vitamin A. ATRA is a ligand of the retinoid acid and retinoid x receptors (RAR and RXR). ATRA has been shown to work in synergy with NGF to accelerate neurite outgrowth and functional recovery (Hernández-Pedro et al. 2008; Palencia et al. 2014). Moreover, it has been shown that ATRA and NGF stimulate each other (Palencia et al. 2014). It is perhaps little surprise that ATRA has shown some effectiveness as a regenerative therapy for a number of acquired axonal neuropathies such as chemotherapy-induced neuropathy (Arrieta et al. 2011). Given concerns for significant side effects with high doses of

ATRA, it is unclear if it'll ever be used in regenerative trials of peripheral neuropathies.

Coenzyme Q10 has an important role in mitochondrial metabolism. In addition, Coenzyme Q10 has been shown to be a potent antioxidant agent (Lance et al. 2012; Lee et al. 2012; Liu et al. 2016). This suggests that Coenzyme Q10 may be an effective agent in mitigating oxidative damage that is key to the pathogenesis of a number of neuropathies. Type II diabetic mice who were given Coenzyme Q10 were resistant to the onset of diabetic neuropathy compared to those not treated with Coenzyme Q10 (Shi et al. 2013). Interestingly, in type II diabetic patients, Coenzyme Q10 levels were shown to be increased compared to healthy individuals (Shen and Pierce 2015). It is thought that this is an adaptive response to circumvent the increased oxidative stress associated with hyperglycemia. The findings of clinical trials that have explored what effectiveness Coenzyme Q10 supplements have remain largely inconclusive (Shen and Pierce 2015).

Vitamin D and its bioactive molecule (calcitriol) stimulate vitamin D receptors. This leads to increased synthesis of proregenerative proteins such as NGF, BDNF, and neurotrophins 2 and 4 (Chabas et al. 2008, 2013). This suggests that Vitamin D has a role in promoting neuroprotection and regeneration. Indeed, it has been shown that Vitamin D deficiency induces neuropathy (Maser et al. 2015; Abdelsadek et al. 2018). Moreover, patients with painful diabetic neuropathy demonstrated improving symptoms following intramuscular injections of Vitamin D (Basit et al. 2016), but unclear if Vitamin D will have any role in regenerative therapies if patients do not show any evidence of Vitamin D deficiency.

6.6 Traditional Chinese Medicine

A number of traditional Chinese medicines are thought to have neuroprotective and proregenerative properties. The Ginsenoside and *Ginkgo biloba* extract are two substances that have recently gained interest as potential regenerative therapies for acquired axonal neuropathies.

In vitro evidence has shown that Ginsenoside (an extract from the ginseng plant) may help ameliorate oxidative stress induced by hyperglycemia (Bai et al. 2018; Zhou et al. 2019). Moreover, in animal models of peripheral nerve injury, the administration of Ginsenoside led to improvements in histological and functional outcome assessments compared to controls (Huo et al. 2015; Wang et al. 2015b). In addition, those treated with Ginsenoside demonstrated enhanced higher concentrations of NGF at all doses tested (Huo et al. 2015). Although these studies suggest that Ginsenoside may have a therapeutic role in acquired axonal neuropathies, further validation studies and experiments exploring mechanisms are needed before clinical studies can take place.

Ginkgo biloba extract has been shown to have neuroprotective effects in preclinical models (Jang et al. 2012; Zhu et al. 2015). This is thought to be mediated through antioxidative and vasodilator activity (Jang et al. 2012; Zhu et al. 2015). By extension, this may protect against pathological processes associated with the

progression of neuropathies. Administration of *Ginkgo biloba* has been shown to enhance neural regeneration and functional recovery in animal models of nerve injury (Jang et al. 2012; Zhu et al. 2015). In addition, a number of clinical studies have demonstrated that diabetic patients who received *Ginkgo biloba* demonstrated an improvement in symptoms associated with neuropathy (Zhang et al. 2013). However, this was not associated with concurrent improvement in electrodiagnostic assessments of nerve function (Zhang et al. 2013), which raises the possibility that the clinical improvements may be due to placebo effects. Further studies are needed to explore potential regenerative benefits of *Ginkgo biloba* in acquired axonal neuropathy.

6.7 Miscellaneous Drugs

Fusadil is a vasodilator and Rho-kinase inhibitor. Through the inhibition of Rho, Fusadil prevents the collapse of newly established growth cones (Madura et al. 2007; Bota and Fodor 2019). Moreover, it has been shown that Fusadil antagonizes the migration of neutrophils to areas of nerve damage (Madura et al. 2007; Bota and Fodor 2019). This is thought to prevent the onset of neuropathic pain and promote neuronal regeneration (Madura et al. 2007). Moreover, intraperitoneal injection of Fusadil in type II diabetic mice significantly improved signs of peripheral neuropathy (Arita et al. 2009; Matoba et al. 2013).

Agmatine sulfate is categorized as an aminoguanidine. Agmatine sulfate is a ligand for multiple molecular targets and it has been shown to accelerate neural regeneration in animal models (Surmelioglu et al. 2017). The exact cellular and molecular mechanisms that are responsible for these observations remain largely unknown. A systematic review of clinical trials that deployed Agmatine sulfate as an oral supplement in patients with diabetic and chemotherapy-induced neuropathies found that this agent improved clinical symptoms and electrodiagnostic measures of nerve function (Fairbanks et al. 2000; Donertas et al. 2018).

CDP-Coline is a naturally occurring compound that is found in human cells. CDP-Coline has been shown to improve nerve regeneration and nociceptive threshold in type II diabetic mice (Kamei et al. 2008). Recent evidence suggests this is attributable to anti-inflammatory and anti-apoptotic properties that disrupt the biological progression of neuropathies (Zhou et al. 2012). In animal models of nerve injury, administration of CDP-Coline significantly improved neural regeneration and functional recovery (Aslan et al. 2011; Bota and Fodor 2019). Clinical studies that establish what effect CDP-Coline has in the clinical management of neuropathies is awaited.

4-Methylcatechol is a nonherbal alkaloid. This agent has been shown to significantly improve neural regeneration and functional recovery in mice with resiniferatoxin-induced neuropathy and sciatic nerve crush injury (Hanaoka et al. 1994; Hsieh et al. 2008, 2009). This was correlated with a significant increase in the expression of NGF, GDNF, and phosphorylated Trk (Hsieh et al. 2008). Similarly, Berberin (a herbal alkaloid) has been shown to have similar effects as NGF,

demonstrating neuroprotective and proregenerative effects in animal models of diabetic neuropathy and neural injury (Zan et al. 2017; Zhang et al. 2018a). Clinical studies that investigate the efficacy of these agents in axonal neuropathies are not well documented.

COMP-Angiopoietin-1 is a drug that has been shown to promote angiogenesis and neural regeneration (Kosacka et al. 2012). In type II diabetic mice, it has been shown to improve the expression of pro-regenerative proteins and improved axonal morphology (Kosacka et al. 2012). Activation of neuronal p38, MAPK, and Akt is thought to be responsible for these observations (Kosacka et al. 2012). In addition, improved neuronal uptake of glucose was reported in those mice treated with COMP-Angiopoietin-1 (Kosacka et al. 2012). Through similar molecular mechanisms, Magnesium has been shown to improve axonal regeneration in animal models of nerve injury (Pan et al. 2011). However, studies administration of Magnesium in patients with neuropathies remain inconclusive (Wesselink et al. 2018; Zhang et al. 2018b).

Chitooligosaccharides result from the degradation of chitosan. This obtained from the shells of crustaceans which have well documented neuroprotective and neuroregenerative effects in animal models of nerve injury (Gong et al. 2009; Jiang et al. 2009). Moreover, it has been demonstrated that Chitooligosaccharides promote the proliferation of pancreatic β cells and therefore glucose control (Liu et al. 2007; Karadeniz et al. 2010). By extension, Chitooligosaccharides has the capacity to protect against the metabolic dysregulation caused by hyperglycemia. Together, this suggests that Chitooligosaccharides could protect against the progression of diabetic neuropathy and promote regeneration, but proper preclinical and clinical studies are needed.

Gangliosides are important components of cell membranes. Nerve injured rodents treated with gangliosides demonstrated improved nerve regeneration and functional outcomes compared to controls (Sobeski et al. 2001; Ribeiro et al. 2008). Moreover, there is extensive electrophysiological and functional evidence that suggest improved nerve recovery in animal models of neuropathy (Ilyas and Chen 2007). Results from randomized controlled trials suggest that treatment of diabetic neuropathy with gangliosides leads to modest improvements in symptoms and some electrophysiological measurements of nerve function (Mallik et al. 2017).

Triptolide is an herbal drug which has been explored as a potential treatment for diabetic neuropathy (Wang et al. 2017). Triptolide has demonstrated antioxidative effects and modulates the inflammatory response (Ziaei and Halaby 2016); both processes are key to the progression of a number of neuropathies. Administration of Triptolide in animal models of nerve injury has also been shown to lead to accelerated axonal regeneration and improved functional outcomes (Zhang et al. 2014). Together, this warranted the recent clinical explorations of Triptolide as a therapy for a number of neuropathies including diabetic neuropathy (Snyder et al. 2016). While these initial results appear promising, randomized controlled clinical trials are awaited.

Resveratrol is an herbal drug. Increased activation of p300 acetyl-transferase-mediated VEGF in the neuronal cell body was demonstrated in nerve injured rats

treated with Resveratrol compared to controls (Ding et al. 2018). Moreover, in type II diabetic mice treated with Resveratrol, higher concentrations of neurotrophic factors such as NGF were reported (Ozturk et al. 2017). Together, these studies suggest further exploration of Resveratrol as a potential regenerative therapy for acquired axonal neuropathies is warranted.

Curcumin is an agent that can be isolated from the herb turmeric. Curcumin has been shown in a number of experimental settings to have anti-inflammatory and antioxidant effects (Menon and Sudheer 2007). As a result, curcumin has gained interest as a potential therapeutic agent in the treatment of a number of neuropathies (Banafshe et al. 2014; Lv et al. 2018). Type II diabetic mice that received intraperitoneal injections of curcumin demonstrated improved symptoms compared to controls (Banafshe et al. 2014; Lv et al. 2018). In addition, curcumin has been shown to increase axonal regeneration and improved functional recovery (Ma et al. 2013, 2016b). It is thought that these observations are at least partially attributable to curcumin mediated downregulation of TNF- α , increased expression of JNK, reduction in caspase 3, and an increase in intracellular cAMP (Banafshe et al. 2014; Lv et al. 2018).

In summary, a wide range of regenerative therapies for acquired axonal neuropathies have been reported. The number of therapies that have tested is overwhelming and the review provided here is not exhaustive. The cellular and molecular mechanisms that underpin the proregenerative properties of these therapeutic agents cover a broad spectrum; some remain to be discovered. The efficacy of these agents has been documented in preclinical models but most await validation in multiple laboratories and translation to clinical therapies remain challenging. This is partly due to high doses needed for efficacy that can't be attained in humans without side effects. Another challenge is the delivery of these therapeutic agents to anatomical regions affected by the underlying neuropathy.

7 Strategies to Deliver Regenerative Therapies for Acquired Axonal Neuropathies

Many of these therapies have demonstrated promising results in vitro with significant improvements in neurite length and neuroprotection. However, a major challenge encountered is the poor ability of regenerating nerves to find their target. For example, in a Stage III clinical trial that investigated the use of NGF as a treatment for diabetic neuropathy, a number of dose-limiting side effects were reported; enhanced pain due to proliferations of ENF's and gastric disturbance were among the most commonly reported adverse effects (Apfel 2002). These observations are attributable to poor specificity of regenerative pathfinding leading to the formation of inappropriate functional connections. This antagonizes the ability of regenerating nerves to reintegrate into the neural circuitry. Therefore, research has been focused on how to maximize the therapeutic benefit of regenerative therapies by improving the specificity of target reinnervation.

Intravenous, oral, and/or intramuscular administration of small therapeutic molecules in humans is often associated with a number of off target effects. Therefore, research has been focused onto developing strategies to improve the specificity of drug delivery to the area affected by the neuropathy. The degeneration of ENF's is a key feature of many acquired axonal neuropathies. Recovery depends upon creating an environment that will facilitate regeneration of these damaged fibers toward the skin surface and epidermis. As a result, topical delivery of therapeutic agents appears to be the ideal method to promote specific and faster reinnervation.

However, the properties of many of the discussed regenerative therapies do not lend themselves toward this goal of topical application. The transdermal delivery of proteins is notoriously challenging due to their physicochemical properties. As a result, a number of approaches have been reported in an attempt to circumvent these challenges such as carrier peptides, iontophoresis, ultrasound, and nanopatches. More recently, gene therapy using nonreplicating viruses has emerged as a method to deliver regenerative therapies to the PNS.

8 Transdermal Drug Delivery

8.1 Cell-Penetrating Carrier Peptides

Cell-penetrating carrier peptides are generally short peptides not exceeding 30 residues. These carrier peptides have the capacity to universally cross cell membrane with minimal toxicity. They achieve this through interactions with energy independent and/or dependent mechanisms and become internalized within the target cell. Importantly, this process is largely independent of the ability of cellular receptors recognizing the peptides. The most widely reported peptides have a slight positive charge and often have a primary or secondary amphipathic characteristic in order to facilitate internalization at the target cell.

Cell-penetrating carrier peptides have been utilized to rapidly deliver a number of proteins across the skin within minutes of application (Benson 2005; Landowski et al. 2016). Moreover, this delivery is highly localized and the direct application results in fewer effects extrinsic to the target area (Benson 2005). Moreover, direct application enhances bioavailability to the affected area. The most widely reported cell penetrating carrier peptide is the HIV-1-derived peptide: TAT (Zhang et al. 2012). TAT has been widely deployed for its cell penetrating properties and has been used to successfully deliver a number of large proteins such as GFP (Hou et al. 2007), epidermal growth factor (Jin et al. 2014) and acidic fibroblast growth factor (Zheng et al. 2015).

8.2 Iontophoresis

Iontophoresis is a noninvasive method that has been used for transdermal drug delivery. Iontophoresis allows drug delivery through the application of a voltage gradient on the skin. The drug molecules traverse the stratum corneum (outermost

layer of the epidermis) by electrophoresis and electroosmosis. The electric field has the added effect of increasing the permeability of the skin making it easier for the drug molecule to reach the intended target: the ENFs. Iontophoresis has been deployed in clinical and experimental settings to treat a number of conditions. For example, iontophoresis of the local anesthetics lidocaine and epinephrine has been patented for local dermal anesthesia (Iontocaine[®]) (Prausnitz and Langer 2008). This has the advantage of not requiring needle injection while also maximizing bioavailability.

Iontophoresis has also been used in synergy with other delivery techniques (Karande et al. 2004). Since iontophoresis supplies a driving force for the delivery of drug molecules, its efficacy can be significantly increased when used in concurrence with a technique that increases skin permeability and/or penetration. Perhaps one of the best examples of this approach was the combination of iontophoresis and microdermabrasion to maximize the transdermal delivery of 5-aminolaevulinic acid. Concentrations of 5-aminolaevulinic acid were 15 times higher at the target site compared to when iontophoresis was used in isolation (Fang et al. 2004). Together, these observations warrant further exploration of iontophoresis as a method to deliver regenerative therapies for acquired axonal neuropathies.

8.3 Nanopatch

The Nanopatch[™] is a specialized system for transdermal drug delivery and has been extensively trialed in the delivery of a number of vaccines (Prow et al. 2010). In essence, this system is an adaptation of the traditional injection. Consisting of a number of fine-tapering needles, the nanopatch allows painless application to the skin. This method has a number of benefits; it allows accurate, highly localized application of the drug to the affected area (Fernando et al. 2010). This may allow the use of lower doses therefore making any treatment more cost effective. However, application of a nanopatch in distal anatomical locations that are heavily involved with the interaction of our environment such as the fingertips affords practical challenges.

8.4 Ultrasound

Ultrasound has a wide number of uses in diagnosing and treating PNS pathologies (see chapter ► “[Clinical Outcome Measures Following Peripheral Nerve Repair](#)”). In the clinical setting of PN, ultrasound may offer a novel method for the delivery of drug molecules.

Cavitation ultrasound involves the application of low frequency ultrasound to facilitate the formation of cavitation bubbles within the applied solution (Prausnitz and Langer 2008). Once the bubbles reach the skin surface, they collapse. The resultant shearing force disrupts the stratum corneum leading to the injection of microjets of fluid into the epidermis.

This method has been used successfully to deliver large molecules into the skin. Moreover, it an FDA-approved system has incorporated this method to enhance and accelerate the delivery of the topical anesthetic lidocaine (Landowski et al. 2016). A similar system may benefit the transdermal delivery of drugs to promote regeneration in PN's.

8.5 Skin Permeability Considerations

All of these methods of transdermal delivery depend upon sufficient skin permeability. This can be achieved through disruption or removal of the epidermis.

A number of approaches have been reported to achieve this. Abrasion of the stratum corneum through microdermabrasion (abrasion of the skin using microcrystals) or tape stripping has been shown to increase the flux of smaller molecules into the skin several fold (Fang et al. 2004; Fujimoto et al. 2005). Tape stripping involves and removal of adhesive tape (approximately 20 cycles) significantly increases skin permeability (Fang et al. 2004; Fujimoto et al. 2005). These approaches should be considered when developing topical drug delivery systems since they have demonstrated good efficacy and are practicable in the clinical setting.

A number of transdermal delivery methods remain impractical for the delivery of small molecules or drugs to large areas of skin affected by PN (for example, whole hands and feet) (Landowski et al. 2016). A likely solution is the combination of delivery systems to improve the transdermal application of drugs for PN.

9 Gene Therapy

Nonreplicating Herpes Simplex Virus (HSV) vectors have emerged as promising candidates for the delivery of therapeutic transgenes to the PNS. This interest has been stimulated by experimental findings which have demonstrated a high rate of infectivity of the virus in the dorsal root ganglia and trigeminal ganglia (Glorioso and Fink 2004; Simonato et al. 2013). In addition, HSV remains present in sensory neurons in a nonintegrated state permanently (Glorioso and Fink 2004; Simonato et al. 2013). This is thought to mimic viral latency. Inoculation of the skin with the HSV vector has been shown to allow specific delivery of therapeutic agents to the PNS (Hao et al. 2006; Zhou et al. 2008; Lau et al. 2012). This therapeutic approach has the benefit of minimizing off-target effects. Moreover, this approach mitigates many of the practical challenges associated with transdermal drug delivery.

Indeed, this method has been employed to deliver neuroprotective and neuroregenerative molecules such as NGF, EPO, and neurotrophin-3 to the PNS (Goss et al. 2002; Chattopadhyay et al. 2003, 2004, 2007, 2009; Wu et al. 2012). This prevented the progression of neuropathy secondary to chemotherapeutic drugs, diabetes, or treatment with pyridoxine in rodent models (Goss et al. 2002; Chattopadhyay et al. 2003, 2004, 2007, 2009; Wu et al. 2012). Clinical trials

exploring the efficacy of the viral delivery of regenerative therapies for acquired axonal neuropathies are currently underway.

10 Conclusions

A diverse range of insults lead to PN. However, the cellular and molecular mechanisms that underpin the pathogenesis of PN can be ultimately converged onto one or more of the following six mechanisms: metabolic dysregulation, covalent modifications, altered mitochondrial and endoplasmic reticulum function leading to excess production of reactive oxygen species, altered intracellular and inflammatory signaling, and slowed axonal transport and channelopathy. A number of regenerative therapies have shown in animal models of PN to disrupt one or more of these processes. Effective clinical translation of these findings is likely to involve improvements in outcome measures and in the delivery of regenerative therapies to their target area.

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References

- Abdelsadek SE, El Saghier EO, Abdel Raheem SI (2018) Serum 25(OH) vitamin D level and its relation to diabetic peripheral neuropathy in Egyptian patients with type 2 diabetes mellitus. *Egypt J Neurol Psychiatr Neurosurg* 54(1):36–36
- Acharjee S, Noorbakhsh F, Stemkowski P, Olechowski C, Cohen E, Ballanyi K, Kerr B, Pardo C, Smith P, Power C (2010) HIV-1 viral protein R causes peripheral nervous system injury associated with in vivo neuropathic pain. *FASEB J* 24:4343–4353
- Adelsberger H, Quasthoff S, Grosskreutz J, Lepier A, Eckel F, Lersch C (2000) The chemotherapeutic oxaliplatin alters voltage-gated Na⁺ channel kinetics on rat sensory neurons. *Eur J Pharmacol* 406(1):25–32
- Allos BM (2001) *Campylobacter jejuni* infections: update on emerging issues and trends. *Clin Infect Dis* 32(8):1201–1206
- Anand P (2004) Neurotrophic factors and their receptors in human sensory neuropathies. *Prog Brain Res* 146:477–492
- Andersson G, Oradd G, Sultan F, Novikov LN (2018) In vivo diffusion tensor imaging, diffusion kurtosis imaging, and tractography of a sciatic nerve injury model in rat at 9.4T. *Sci Rep* 8(1):12911
- Antoniou T, Weisdorf T, Gough K (2003) Symptomatic hyperlactatemia in an HIV-positive patient: a case report and discussion. *CMAJ* 168(2):195–198
- Apfel SC (2002) Nerve growth factor for the treatment of diabetic neuropathy: what went wrong, what went right, and what does the future hold? *Int Rev Neurobiol* 50:393–413
- Argyriou AA, Kyritsis AP, Makatsoris T, Kalofonos HP (2014) Chemotherapy-induced peripheral neuropathy in adults: a comprehensive update of the literature. *Cancer Manag Res* 6(1):135–147
- Arita R, Hata Y, Nakao S, Kita T, Miura M, Kawahara S, Zandi S, Almulki L, Tayyari F, Shimokawa H, Hafezi-Moghadam A, Ishibashi T (2009) Rho kinase inhibition by fasudil ameliorates diabetes-induced microvascular damage. *Diabetes* 58(1):215–226

- Arrieta Ó, Hernández-Pedro N, Fernández-González-Aragón MC, Saavedra-Pérez D, Campos-Parra AD, Ríos-Trejo MÁ, Cerón-Lizárraga T, Martínez-Barrera L, Pineda B, Ordóñez G, Ortiz-Plata A, Granados-Soto V, Sotelo J (2011) Retinoic acid reduces chemotherapy-induced neuropathy in an animal model and patients with lung cancer. *Neurology* 77(10):987–995
- Aslan E, Kocaeli H, Bekar A, Tolunay S, Ulus IH (2011) CDP-choline and its endogenous metabolites, cytidine and choline, promote the nerve regeneration and improve the functional recovery of injured rat sciatic nerves. *Neurol Res* 33(7):766–773
- Authier FJ, Bassez G, Payan C, Guillevin L, Pawlotsky JM, Degos JD, Gherardi RK, Belec L (2003) Detection of genomic viral RNA in nerve and muscle of patients with HCV neuropathy. *Neurology* 60(5):808–812
- Azhary H, Farooq MU, Bhanushali M, Majid A, Kassab MY (2010) Peripheral neuropathy: differential diagnosis and management. *Am Fam Physician* 81(7):887–892
- Bai L, Gao J, Wei F, Zhao J, Wang D, Wei J (2018) Therapeutic potential of ginsenosides as an adjuvant treatment for diabetes. *Front Pharmacol* 9:423–423
- Banafshe HR, Hamidi GA, Nouredini M, Mirhashemi SM, Mokhtari R, Shofierpour M (2014) Effect of curcumin on diabetic peripheral neuropathic pain: possible involvement of opioid system. *Eur J Pharmacol* 723:202–206
- Bargmann CI, Horvitz HR (1991) Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in *C. elegans*. *Neuron* 7(5):729–742
- Barja G, Herrero A (1998) Localization at complex I and mechanism of the higher free radical production of brain nonsynaptic mitochondria in the short-lived rat than in the longevous pigeon. *J Bioenerg Biomembr* 30(3):235–243
- Basit A, Basit KA, Fawwad A, Shaheen F, Fatima N, Petropoulos IN, Alam U, Malik RA (2016) Vitamin D for the treatment of painful diabetic neuropathy. *BMJ Open Diabetes Res Care* 4(1):e000148–e000148
- Beijers AJ, Mols F, Vreugdenhil G (2014) A systematic review on chronic oxaliplatin-induced peripheral neuropathy and the relation with oxaliplatin administration. *Support Care Cancer* 22(7):1999–2007
- Benson HAE (2005) Transdermal drug delivery: penetration enhancement techniques. *Curr Drug Deliv* 2(1):23–33
- Binda D, Cavaletti G, Cornblath DR, Merkies IS, CIPS Group (2015) Rasch-transformed total neuropathy score clinical version (RT-TNSc(c)) in patients with chemotherapy-induced peripheral neuropathy. *J Peripher Nerv Syst* 20(3):328–332
- Bodner A, Toth PT, Miller RJ (2004) Activation of c-Jun N-terminal kinase mediates gp120IIIB- and nucleoside analogue-induced sensory neuron toxicity. *Exp Neurol* 188(2):246–253
- Bota O, Fodor L (2019) The influence of drugs on peripheral nerve regeneration. *Drug Metab Rev* 51(3):266–292
- Boubaker K, Flepp M, Sudre P, Furrer H, Haensel A, Hirschel B, Boggian K, Chave JP, Bernasconi E, Egger M, Opravil M, Rickenbach M, Francioli P, Telenti A (2001) Hyperlactatemia and antiretroviral therapy: the Swiss HIV Cohort Study. *Clin Infect Dis* 33(11):1931–1937
- Brown TJ, Pittman AL, Monaco GN, Benscoter BJ, Mantravadi AV, Akst LM, Jones KJ, Foecking EM (2013) Androgen treatment and recovery of function following recurrent laryngeal nerve injury in the rat. *Restor Neurol Neurosci* 31(2):169–176
- Brownlee M, Pongor S, Cerami A (1983) Covalent attachment of soluble proteins by non-enzymatically glycosylated collagen. Role in the in situ formation of immune complexes. *J Exp Med* 158(5):1739–1744
- Cacoub P, Renou C, Rosenthal E, Cohen P, Loury I, Loustaud-Ratti V, Yamamoto AM, Camproux AC, Hausfater P, Musset L, Veyssier P, Raguin G, Piette JC, Amoura Z, Boissonnas A, Cacoub P, Camproux AC, Carrat F, Chapelon-Abric C, Chemlal K, Cosserat J, De Gennes C, Francès C, Ginsburg C, Godeau P, Hausfater P, Karmochkine M, Le Moing V, Musset L, Papo T, Piette JC, Poinson Y, Raguin G, Simon J, Wechsler B, Yamamoto AM, Ziza JM, Bernard N, Lacoste D, Loury I, Morlat P, Bouchard O, Micoud M, Chambourlier P, Renou C, Cohen P, Guillevin L, De Korwin JD, Wahl D, Devars Du Mayne JF, Dupont C, Hanslik T, Durand JM, Kaplanski G, Fior R, Galanaud R, Geffray L, Veyssier P, Goujard C, Hayek R, Laurichesse H, Le Lostec Z, Lunel-

- Fabiani F, Loustaud-Ratti V, Ouzan D, Perronne C, Raabe JJ, Wang A, Rosenthal E (2000) Extrahepatic manifestations associated with hepatitis C virus infection: a prospective multicenter study of 321 patients. *Medicine* 79(1):47–56
- Calcutt NA, Jolivald CG, Fernyhough P (2008) Growth factors as therapeutics for diabetic neuropathy. *Curr Drug Targets* 9(1):47–59
- Cashman CR, Höke A (2015) Mechanisms of distal axonal degeneration in peripheral neuropathies. *Neurosci Lett* 596:33–50
- Caudy M, Bentley D (1986) Pioneer growth cone steering along a series of neuronal and non-neuronal cues of different affinities. *J Neurosci* 6(6):1781–1795
- Cavaletti G (2014) Chemotherapy-induced peripheral neurotoxicity (CIPN): what we need and what we know. *J Peripher Nerv Syst* 19(2):66–76
- Chabas J-F, Alluin O, Rao G, Garcia S, Lavaut M-N, Risso JJ, Legre R, Magalon G, Khrestchatsky M, Marqueste T (2008) Vitamin D2 potentiates axon regeneration. *J Neurotrauma* 25(10):1247–1256
- Chabas J-F, Stephan D, Marqueste T, Garcia S, Lavaut M-N, Nguyen C, Legre R, Khrestchatsky M, Decherchi P, Feron F (2013) Cholecalciferol (vitamin D3) improves myelination and recovery after nerve injury. *PLoS One* 8(5):e65034
- Chan KM, Gordon T, Zochodne DW, Power HA (2014) Improving peripheral nerve regeneration: from molecular mechanisms to potential therapeutic targets. *Exp Neurol* 261:826–835
- Chattopadhyay M, Goss J, Lacomis D, Goins WC, Glorioso JC, Mata M, Fink DJ (2003) Protective effect of HSV-mediated gene transfer of nerve growth factor in pyridoxine neuropathy demonstrates functional activity of trkA receptors in large sensory neurons of adult animals. *Eur J Neurosci* 17(4):732–740
- Chattopadhyay M, Goss J, Wolfe D, Goins WC, Huang S, Glorioso JC, Mata M, Fink DJ (2004) Protective effect of herpes simplex virus-mediated neurotrophin gene transfer in cisplatin neuropathy. *Brain* 127(Pt 4):929–939
- Chattopadhyay M, Mata M, Goss J, Wolfe D, Huang S, Glorioso JC, Fink DJ (2007) Prolonged preservation of nerve function in diabetic neuropathy in mice by herpes simplex virus-mediated gene transfer. *Diabetologia* 50(7):1550–1558
- Chattopadhyay M, Walter C, Mata M, Fink DJ (2009) Neuroprotective effect of herpes simplex virus-mediated gene transfer of erythropoietin in hyperglycemic dorsal root ganglion neurons. *Brain* 132(Pt 4):879–888
- Chaunu MP, Ratnahirana H, Raphael M, Hénin D, Leport C, Brun-Vezinet F, Léger JM, Brunet P, Hauw JJ (1989) The spectrum of changes on 20 nerve biopsies in patients with HIV infection. *Muscle Nerve* 12(6):452–459
- Cheng Y, Liu J, Luan Y, Liu Z, Lai H, Zhong W, Yang Y, Yu H, Feng N, Wang H, Huang R, He Z, Yan M, Zhang F, Sun YG, Ying H, Guo F, Zhai Q (2019) Sarm1 gene deficiency attenuates diabetic peripheral neuropathy in mice. *Diabetes* 68(11):2120–2130
- Clements RS Jr, Bell DS (1982) Diagnostic, pathogenetic, and therapeutic aspects of diabetic neuropathy. *Spec Top Endocrinol Metab* 3:1–43
- Cohen S, Levi-Montalcini R, Hamburger V (1954) A nerve growth-stimulating factor isolated from sarcomas 37 and 180. *Proc Natl Acad Sci U S A* 40(10):1014–1018
- Coleman MP, Freeman MR (2010) Wallerian degeneration, wld(s), and nmnat. *Annu Rev Neurosci* 33:245–267
- Cornblath DR, McArthur JC (1988) Predominantly sensory neuropathy in patients with AIDS and AIDS-related complex. *Neurology* 38(5):794–796
- Cornblath DR, Chaudhry V, Carter K, Lee D, Seysedadr M, Miernicki M, Joh T (1999) Total neuropathy score: validation and reliability study. *Neurology* 53(8):1660–1664
- de La Monte SM, Gabuzda DH, Ho DD, Brown RH Jr, Hedley-Whyte ET, Schooley RT, Hirsch MS, Bhan AK (1988) Peripheral neuropathy in the acquired immunodeficiency syndrome. *Ann Neurol* 23(5):485–492
- Deckwerth TL, Johnson EM Jr (1994) Neurites can remain viable after destruction of the neuronal soma by programmed cell death (apoptosis). *Dev Biol* 165(1):63–72

- Di Stefano G, Di Lionardo A, Galosi E, Truini A, Cruccu G (2019) Acetyl-L-carnitine in painful peripheral neuropathy: a systematic review. *J Pain Res* 12:1341–1351
- Dickerman RD, Kramer E, Pertusi R, McConathy WJ (1997) Peripheral neuropathy and testosterone. *Neurotoxicology* 18(2):587–588
- Ding Z, Cao J, Shen Y, Zou Y, Yang X, Zhou W, Guo Q, Huang C (2018) Resveratrol promotes nerve regeneration via activation of p300 acetyltransferase-mediated VEGF signaling in a rat model of sciatic nerve crush injury. *Front Neurosci* 12:341
- Donertas B, Unel CC, Erol K (2018) Cannabinoids and agmatine as potential therapeutic alternatives for cisplatin-induced peripheral neuropathy. *J Exp Pharmacol* 10:19–28
- Du Z-j, Wang L, Lei D-l, Liu B-l, Cao J, Zhang P, Ma Q, Surgery M (2011) Nerve growth factor injected systemically improves the recovery of the inferior alveolar nerve in a rabbit model of mandibular distraction osteogenesis. *Br J Oral Maxillofac Surg* 49(7):557–561
- Duran-Jimenez B, Dobler D, Moffatt S, Rabbani N, Streuli CH, Thornalley PJ, Tomlinson DR, Gardiner NJ (2009) Advanced glycation end products in extracellular matrix proteins contribute to the failure of sensory nerve regeneration in diabetes. *Diabetes* 58(12):2893–2903
- Dyck PJ, Kratz KM, Karnes JL, Litchy WJ, Klein R, Pach JM, Wilson DM, O'Brien PC, Melton LJ III (1993) The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester diabetic neuropathy study. *Neurology* 43(4):817–824
- Ebadi M, Bashir RM, Heidrick ML, Hamada FM, Refaey HEL, Hamed A, Helal G, Baxi MD, Cerutti DR, Lassi NK (1997) Neurotrophins and their receptors in nerve injury and repair. *Neurochem Int* 30(4-5):347–374
- El-Fatraty BM, Ibrahim OM, Hussien FZ, Mostafa TM (2018) Role of metformin in oxaliplatin-induced peripheral neuropathy in patients with stage III colorectal cancer: randomized, controlled study. *Int J Color Dis* 33(12):1675–1683
- Erbas O, Taskiran A, Oltulu F, Yavasoglu A, Bora S, Bilge O, Cinar BP, Peker G (2017) Oxytocin provides protection against diabetic polyneuropathy in rats. *Neurol Res* 39(1):45–53
- Escobar-Jimenez F (2002) Efficacy and safety of sildenafil in men with type 2 diabetes mellitus and erectile dysfunction. *Med Clin (Barc)* 119(4):121–124
- Esteghamati A, Eskandari D, Mirmiranpour H, Noshad S, Mousavizadeh M, Hedayati M, Nakhjavani M (2013) Effects of metformin on markers of oxidative stress and antioxidant reserve in patients with newly diagnosed type 2 diabetes: a randomized clinical trial. *Clin Nutr* 32(2):179–185
- Fairbanks CA, Schreiber KL, Brewer KL, Yu CG, Stone LS, Kitto KF, Nguyen HO, Grocholski BM, Shoeman DW, Kehl LJ, Regunathan S, Reis DJ, Yezierski RP, Wilcox GL (2000) Agmatine reverses pain induced by inflammation, neuropathy, and spinal cord injury. *Proc Natl Acad Sci U S A* 97(19):10584–10589
- Fang JY, Lee WR, Shen SC, Fang YP, Hu CH (2004) Enhancement of topical 5-aminolaevulinic acid delivery by erbium:YAG laser and microdermabrasion: a comparison with iontophoresis and electroporation. *Br J Dermatol* 151(1):132–140
- Fang T, Shao Y, Oswald T, Lineaweaver WC, Zhang F (2013) Effect of sildenafil on peripheral nerve regeneration. *Ann Plast Surg* 70(1):62–65
- Fernando GJ, Chen X, Prow TW, Crichton ML, Fairmaid EJ, Roberts MS, Frazer IH, Brown LE, Kendall MA (2010) Potent immunity to low doses of influenza vaccine by probabilistic guided micro-targeted skin delivery in a mouse model. *PLoS One* 5(4):e10266
- Ferri C, Zignego AL, Pileri SA (2002) Cryoglobulins. *J Clin Pathol* 55(1):4–13
- Ferrier J, Pereira V, Busserolles J, Authier N, Balayssac D (2013) Emerging trends in understanding chemotherapy-induced peripheral neuropathy. *Curr Pain Headache Rep* 17(10):364
- Finn JT, Weil M, Archer F, Siman R, Srinivasan A, Raff MC (2000) Evidence that wallerian degeneration arid localized axon degeneration induced by local neurotrophin deprivation do not involve caspases. *J Neurosci* 20(4):1333–1341
- Fujimoto S, Amako K (1990) Guillain-Barré syndrome and campylobacter jejuni infection. *Lancet* 335(8701):1350

- Fujimoto T, Shirakami K, Tojo K (2005) Effect of microdermabrasion on barrier capacity of stratum corneum. *Chem Pharm Bull* 53(8):1014–1016
- Gabbay KH, Merola LO, Field RA (1966) Sorbitol pathway: presence in nerve and cord with substrate accumulation in diabetes. *Science* 151(3707):209–210
- Gao WQ, Shinsky N, Ingle G, Beck K, Elias KA, Powell-Braxton L (1999) IGF-I deficient mice show reduced peripheral nerve conduction velocities and decreased axonal diameters and respond to exogenous IGF-I treatment. *J Neurobiol* 39(1):142–152
- Geisler S, Doan RA, Strickland A, Huang X, Milbrandt J, DiAntonio A (2016) Prevention of vincristine-induced peripheral neuropathy by genetic deletion of SARM1 in mice. *Brain* 139(Pt 12):3092–3108
- Geisler S, Doan RA, Cheng GC, Cetinkaya-Fisgin A, Huang SX, Hoke A, Milbrandt J, DiAntonio A (2019) Vincristine and bortezomib use distinct upstream mechanisms to activate a common SARM1-dependent axon degeneration program. *JCI Insight* 4(17):e129920
- Gerdts J, Summers DW, Sasaki Y, DiAntonio A, Milbrandt J (2013) Sarm1-mediated axon degeneration requires both SAM and TIR interactions. *J Neurosci* 33(33):13569–13580
- Gerdts J, Brace EJ, Sasaki Y, DiAntonio A, Milbrandt J (2015) SARM1 activation triggers axon degeneration locally via NAD(+) destruction. *Science* 348(6233):453–457
- Glass JD, Brushart TM, George EB, Griffin JW (1993) Prolonged survival of transected nerve fibres in C57BL/Ola mice is an intrinsic characteristic of the axon. *J Neurocytol* 22(5):311–321
- Glorioso JC, Fink DJ (2004) Herpes vector-mediated gene transfer in treatment of diseases of the nervous system. *Annu Rev Microbiol* 58:253–271
- Gong Y, Gong L, Gu X, Ding F (2009) Chitoooligosaccharides promote peripheral nerve regeneration in a rabbit common peroneal nerve crush injury model. *Microsurgery* 29(8):650–656
- Gornstein E, Schwarz TL (2014) The paradox of paclitaxel neurotoxicity: mechanisms and unanswered questions. *Neuropharmacology* 76(Pt A):175–183
- Goss JR, Goins WF, Lacomis D, Mata M, Glorioso JC, Fink DJ (2002) Herpes simplex-mediated gene transfer of nerve growth factor protects against peripheral neuropathy in streptozotocin-induced diabetes in the mouse. *Diabetes* 51(7):2227–2232
- Guillain G (1916) Sur un syndrome de radiculo-nevrite avec hyperalbuminose du liquide cephalorachidien sans reaction cellulaire: remarques sur les caracteres cliniques et graphiques des reflexes tendineux. *Bull Soc Med Hop Paris* 40:1462–1470
- Gümüş B, Kuyucu E, Erbas O, Kazimoglu C, Oltulu F, Bora OA (2015) Effect of oxytocin administration on nerve recovery in the rat sciatic nerve damage model. *J Orthop Surg Res* 10:161–161
- Guo HL, Yang Y, Geng ZP, Zhu LX, Yuan SY, Zhao Y, Gao YH, Fu HJ (1999) The change of insulin-like growth factor-1 in diabetic patients with neuropathy. *Chin Med J* 112(1):76–79
- Gupta M, Singh J, Sood S, Arora B (2003) Mechanism of antinociceptive effect of nimodipine in experimental diabetic neuropathic pain. *Methods Find Exp Clin Pharmacol* 25(1):49–52
- Hahn K, Triolo A, Hauer P, McArthur JC, Polydefkis M (2007) Impaired reinnervation in HIV infection following experimental denervation. *Neurology* 68(16):1251–1256
- Hahn K, Robinson B, Anderson C, Li W, Pardo CA, Morgello S, Simpson D, Nath A (2008) Differential effects of HIV infected macrophages on dorsal root ganglia neurons and axons. *Exp Neurol* 210(1):30–40
- Han Y, Smith MT (2013) Pathobiology of cancer chemotherapy-induced peripheral neuropathy (CIPN). *Front Pharmacol* 4:156
- Hanaoka Y, Ohi T, Furukawa S, Furukawa Y, Hayashi K, Matsukura S (1994) The therapeutic effects of 4-methylcatechol, a stimulator of endogenous nerve growth factor synthesis, on experimental diabetic neuropathy in rats. *J Neurol Sci* 122(1):28–32
- Hao S, Mata M, Glorioso JC, Fink DJ (2006) HSV-mediated expression of interleukin-4 in dorsal root ganglion neurons reduces neuropathic pain. *Mol Pain* 2:6–6
- Hernández-Pedro N, Ordóñez G, Ortiz-Plata A, Palencia-Hernández G, García-Ulloa AC, Flores-Estrada D, Sotelo J, Arrieta OJTR (2008) All-trans retinoic acid induces nerve regeneration and increases serum and nerve contents of neural growth factor in experimental diabetic neuropathy. *Transl Res* 152(1):31–37

- Hershman DL, Unger JM, Crew KD, Minasian LM, Awad D, Moinpour CM, Hansen L, Lew DL, Greenlee H, Fehrenbacher L (2013) Randomized double-blind placebo-controlled trial of acetyl-L-carnitine for the prevention of taxane-induced neuropathy in women undergoing adjuvant breast cancer therapy. *J Clin Oncol* 31(20):2627
- Himeno T, Kamiya H, Naruse K, Harada N, Ozaki N, Seino Y, Shibata T, Kondo M, Kato J, Okawa T, Fukami A, Hamada Y, Inagaki N, Seino Y, Drucker DJ, Oiso Y, Nakamura J (2011) Beneficial effects of exendin-4 on experimental polyneuropathy in diabetic mice. *Diabetes* 60(9):2397–2406
- Höke A, Morris M, Haughey NJ (2009) GPI-1046 protects dorsal root ganglia from gp120-induced axonal injury by modulating store-operated calcium entry. *JPNS* 14(1):27–35
- Hou YW, Chan MH, Hsu HR, Liu BR, Chen CP, Chen HH, Lee HJ (2007) Transdermal delivery of proteins mediated by non-covalently associated arginine-rich intracellular delivery peptides. *Exp Dermatol* 16(12):999–1006
- Hsieh YL, Chiang H, Tseng TJ, Hsieh ST (2008) Enhancement of cutaneous nerve regeneration by 4-methylcatechol in resiniferatoxin-induced neuropathy. *J Neuropathol Exp Neurol* 67(2):93–104
- Hsieh Y-L, Lin W-M, Lue J-H, Chang M-F, Hsieh S-T (2009) Effects of 4-methylcatechol on skin reinnervation: promotion of cutaneous nerve regeneration after crush injury. *J Neuropathol Exp Neurol* 68(12):1269–1281
- Huo DS, Zhang M, Cai ZP, Dong CX, Wang H, Yang ZJ (2015) The role of nerve growth factor in ginsenoside Rg1-induced regeneration of injured rat sciatic nerve. *J Toxicol Environ Health A* 78(21–22):1328–1337
- Hur E-M, Saijilafu, Zhou F-Q (2012) Growing the growth cone: remodeling the cytoskeleton to promote axon regeneration. *Trends Neurosci* 35(3):164–174
- IDF (2014) International Diabetes Federation Diabetes Atlas, vol 4. IDF, Brussels
- Ilyas AA, Chen Z-W (2007) Lewis rats immunized with GM1 ganglioside do not develop peripheral neuropathy. *J Neuroimmunol* 188(1–2):34–38
- Jang CH, Cho YB, Choi CH (2012) Effect of *Ginkgo biloba* extract on recovery after facial nerve crush injury in the rat. *Int J Pediatr Otorhinolaryngol* 76(12):1823–1826
- Jayabalan B, Low LL (2016) Vitamin B supplementation for diabetic peripheral neuropathy. *Singap Med J* 57(2):55–59
- Jennum P, Ibsen R, Kjellberg J (2015) Health, social and economic consequences of polyneuropathy: a controlled national study evaluating societal effects on patients and their partners. *Eur Neurol* 73(1–2):81–88
- Jiang M, Zhuge X, Yang Y, Gu X, Ding F (2009) The promotion of peripheral nerve regeneration by chitooligosaccharides in the rat nerve crush injury model. *Neurosci Lett* 454(3):239–243
- Jin PP, Li FF, Ruan RQ, Zhang L, Man N, Hu Y, Zhou W, Wen LP (2014) Enhanced transdermal delivery of epidermal growth factor facilitated by dual peptide chaperone motifs. *Protein Pept Lett* 21(6):550–555
- Joint United Nations Programme on HIV/AIDS (2010) Global report: UNAIDS report on the global AIDS epidemic. UNAIDS, Geneva
- Jones G, Zhu Y, Silva C, Tsutsui S, Pardo CA, Keppler OT, McArthur JC, Power C (2005) Peripheral nerve-derived HIV-1 is predominantly CCR5-dependent and causes neuronal degeneration and neuroinflammation. *Virology* 334(2):178–193
- Julius D, Basbaum AI (2001) Molecular mechanisms of nociception. *Nature* 413(6852):203–210
- Kamei J, Ohsawa M, Miyata S, Endo K, Hayakawa H (2008) Effects of cytidine 5'-diphosphocholine (CDP-choline) on the thermal nociceptive threshold in streptozotocin-induced diabetic mice. *Eur J Pharmacol* 598(1–3):32–36
- Kamerman PR, Moss PJ, Weber J, Wallace VCJ, Rice ASC, Huang W (2012) Pathogenesis of HIV-associated sensory neuropathy: evidence from in vivo and in vitro experimental models. *J Peripher Nerv Syst* 17(1):19–31
- Kan M, Guo G, Singh B, Singh V, Zochodne DW (2012) Glucagon-like peptide 1, insulin, sensory neurons, and diabetic neuropathy. *J Neuropathol Exp Neurol* 71(6):494–510
- Karadeniz F, Artan M, Kong C-S, Kim S-K (2010) Chitooligosaccharides protect pancreatic β -cells from hydrogen peroxide-induced deterioration. *Carbohydr Polym* 82(1):143–147

- Karande P, Jain A, Mitragotri S (2004) Discovery of transdermal penetration enhancers by high-throughput screening. *Nat Biotechnol* 22(2):192–197
- Karlidag T, Yildiz M, Yalcin S, Colakoglu N, Kaygusuz I, Sapmaz E (2012) Evaluation of the effect of methylprednisolone and N-acetylcysteine on anastomotic degeneration and regeneration of the facial nerve. *Auris Nasus Larynx* 39(2):145–150
- Keilbaugh SA, Hobbs GA, Simpson MV (1997) Effect of 2',3'-dideoxycytidine on oxidative phosphorylation in the PC 12 cell, a neuronal model. *Biochem Pharmacol* 53(10):1485–1492
- Keswani SC, Pardo CA, Cherry CL, Hoke A, McArthur JC (2002) HIV-associated sensory neuropathies. *AIDS* 16(16):2105–2117
- Ketschek A, Jones S, Spillane M, Korobova F, Svitkina T, Gallo G (2015) Nerve growth factor promotes reorganization of the axonal microtubule array at sites of axon collateral branching. *Dev Neurobiol* 75(12):1441–1461
- Kidd JF, Pilkington MF, Schell MJ, Fogarty KE, Skepper JN, Taylor CW, Thorn P (2002) Paclitaxel affects cytosolic calcium signals by opening the mitochondrial permeability transition pore. *J Biol Chem* 277(8):6504–6510
- Kosacka J, Nowicki M, Kloting N, Kern M, Stumvoll M, Bechmann I, Serke H, Bluher M (2012) COMP-angiopoietin-1 recovers molecular biomarkers of neuropathy and improves vascularisation in sciatic nerve of ob/ob mice. *PLoS One* 7(3):e32881
- Lance J, McCabe S, Clancy RL, Pierce J (2012) Coenzyme Q10 – a therapeutic agent. *Medsurg Nurs* 21(6):367–371
- Landowski LM, Dyck PJB, Engelstad J, Taylor BV (2016) Axonopathy in peripheral neuropathies: mechanisms and therapeutic approaches for regeneration. *J Chem Neuroanat* 76:19–27
- Lankford K, Arroyo E, Liu C-N, Somps C, Zorbas M, Shelton D, Evans M, Hurst S, Kocsis J (2013) Sciatic nerve regeneration is not inhibited by anti-NGF antibody treatment in the adult rat. *Neuroscience* 241:157–169
- Lau D, Harte SE, Morrow TJ, Wang S, Mata M, Fink DJ (2012) Herpes simplex virus vector-mediated expression of interleukin-10 reduces below-level central neuropathic pain after spinal cord injury. *Neurorehabil Neural Repair* 26(7):889–897
- Lauria G, Devigili G (2007) Skin biopsy as a diagnostic tool in peripheral neuropathy. *Nat Clin Pract Neurol* 3(10):546–557
- Lavanchy D (2011) Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 17(2):107–115
- Lee BJ, Huang YC, Chen SJ, Lin PT (2012) Coenzyme Q10 supplementation reduces oxidative stress and increases antioxidant enzyme activity in patients with coronary artery disease. *Nutrition* 28(3):250–255
- Lee KA, Park KT, Yu HM, Jin HY, Baek HS, Park TS (2013) Effect of granulocyte colony-stimulating factor on the peripheral nerves in streptozotocin-induced diabetic rat. *Diabetes Metab J* 37(4):286–290
- Lee B-J, Lin J-S, Lin Y-C, Lin P-T (2014) Effects of L-carnitine supplementation on oxidative stress and antioxidant enzymes activities in patients with coronary artery disease: a randomized, placebo-controlled trial. *Nutr J* 13:79–79
- Léger J-M, Guimarães-Costa R, Muntean C (2016) Immunotherapy in peripheral neuropathies. *Neurotherapeutics* 13(1):96–107
- Lehmann HC, Zhang J, Mori S, Sheikh KA (2010) Diffusion tensor imaging to assess axonal regeneration in peripheral nerves. *Exp Neurol* 223(1):238–244
- Leininger GM, Vincent AM, Feldman EL (2004) The role of growth factors in diabetic peripheral neuropathy. *J Peripher Nerv Syst* 9(1):26–53
- Lema MJ, Foley KM, Hausheer FH (2010) Types and epidemiology of cancer-related neuropathic pain: the intersection of cancer pain and neuropathic pain. *Oncologist* 15(Suppl 2):3–8
- Liu B, Liu W-S, Han B-Q, Sun Y-Y (2007) Antidiabetic effects of chitooligosaccharides on pancreatic islet cells in streptozotocin-induced diabetic rats. *World J Gastroenterol* 13(5):725–731

- Liu H-T, Huang Y-C, Cheng S-B, Huang Y-T, Lin P-T (2016) Effects of coenzyme Q10 supplementation on antioxidant capacity and inflammation in hepatocellular carcinoma patients after surgery: a randomized, placebo-controlled trial. *Nutr J* 15(1):85–85
- Lubec D, Müllbacher W, Finsterer J, Mamoli B (1999) Diagnostic work-up in peripheral neuropathy: an analysis of 171 cases. *Postgrad Med J* 75(890):723–727
- Lunn ER, Perry VH, Brown MC, Rosen H, Gordon S (1989) Absence of Wallerian degeneration does not hinder regeneration in peripheral nerve. *Eur J Neurosci* 1(1):27–33
- Luria S, Cohen A, Safran O, Firman S, Liebergall M (2013) Immune system augmentation by glatiramer acetate of peripheral nerve regeneration—crush versus transection models of rat sciatic nerve. *J Reconstr Microsurg* 29(08):495–500
- Lv J, Cao L, Zhang R, Bai F, Wei P (2018) A curcumin derivative J147 ameliorates diabetic peripheral neuropathy in streptozotocin (STZ)-induced DPN rat models through negative regulation AMPK on TRPA1. *Acta Cir Bras* 33(6):533–541
- Ma J, Liu J, Yu H, Wang Q, Chen Y, Xiang L (2013) Curcumin promotes nerve regeneration and functional recovery in rat model of nerve crush injury. *Neurosci Lett* 547:26–31
- Ma J, Liu J, Yu H, Chen Y, Wang Q, Xiang L (2016a) Beneficial effect of metformin on nerve regeneration and functional recovery after sciatic nerve crush injury in diabetic rats. *Neurochem Res* 41(5):1130–1137
- Ma J, Yu H, Liu J, Chen Y, Wang Q, Xiang L (2016b) Curcumin promotes nerve regeneration and functional recovery after sciatic nerve crush injury in diabetic rats. *Neurosci Lett* 610:139–143
- Mack TG, Reiner M, Beirowski B, Mi W, Emanuelli M, Wagner D, Thomson D, Gillingwater T, Court F, Conforti L, Fernando FS, Tarlton A, Andressen C, Addicks K, Magni G, Ribchester RR, Perry VH, Coleman MP (2001) Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. *Nat Neurosci* 4(12):1199–1206
- Madura T, Kubo T, Tanag M, Matsuda K, Tomita K, Yano K, Hosokawa K (2007) The Rho-associated kinase inhibitor fasudil hydrochloride enhances neural regeneration after axotomy in the peripheral nervous system. *Plast Reconstr Surg* 119(2):526–535
- Mallik S, Kallis C, Lunn MPT, Smith AG (2017) Gangliosides for the treatment of diabetic peripheral neuropathy. *Cochrane Database Syst Rev* 2017(6):CD011028
- Martyn CN, Hughes RA (1997) Epidemiology of peripheral neuropathy. *J Neurol Neurosurg Psychiatry* 62(4):310–318
- Maser RE, Lenhard MJ, Pohlrig RT (2015) Vitamin D insufficiency is associated with reduced parasympathetic nerve Fiber function in type 2 diabetes. *Endocr Pract* 21(2):174–181
- Matoba K, Kawanami D, Okada R, Tsukamoto M, Kinoshita J, Ito T, Ishizawa S, Kanazawa Y, Yokota T, Murai N, Matsufuji S, Takahashi-Fujigasaki J, Utsunomiya K (2013) Rho-kinase inhibition prevents the progression of diabetic nephropathy by downregulating hypoxia-inducible factor 1alpha. *Kidney Int* 84(3):545–554
- McArthur JC, Brew BJ, Nath A (2005) Neurological complications of HIV infection. *Lancet Neurol* 4(9):543–555
- McDonald ES, Randon KR, Knight A, Windebank AJ (2005) Cisplatin preferentially binds to DNA in dorsal root ganglion neurons in vitro and in vivo: a potential mechanism for neurotoxicity. *Neurobiol Dis* 18(2):305–313
- Medori R, Autilio-Gambetti L, Monaco S, Gambetti P (1985) Experimental diabetic neuropathy: impairment of slow transport with changes in axon cross-sectional area. *Proc Natl Acad Sci U S A* 82(22):7716–7720
- Menon VP, Sudheer AR (2007) Antioxidant and anti-inflammatory properties of curcumin. *Adv Exp Med Biol* 595:105–125
- Merkies IS, Lauria G, Faber CG (2012) Outcome measures in peripheral neuropathies: requirements through statements. *Curr Opin Neurol* 25(5):556–563
- Merkies IS, Faber CG, Lauria G (2015) Advances in diagnostics and outcome measures in peripheral neuropathies. *Neurosci Lett* 596:3–13

- Migdalís IN, Kalantzís L, Samartzís M, Kalogeropoulou K, Nounopoulos C, Bouloukos A (1995) Insulin-like growth factor-I and IGF-I receptors in diabetic patients with neuropathy. *Diabet Med* 12(9):823–827
- Mironov S, Ivannikov M, Johansson M (2005) $[Ca^{2+}]_i$ signaling between mitochondria and endoplasmic reticulum in neurons is regulated by microtubules - from mitochondrial permeability transition pore to Ca^{2+} -induced Ca^{2+} release. *J Biol Chem* 280:715–721
- Mullokandov EA, Franklin WA, Brownlee M (1994) DNA damage by the glycation products of glyceraldehyde 3-phosphate and lysine. *Diabetologia* 37(2):145–149
- Nahleh Z, Pruemer J, Lafollette J, Sweany S (2010) Melatonin, a promising role in taxane-related neuropathy. *Clin Med Insights Oncol* 4:35–41
- Naudi A, Jove M, Ayala V, Cassanye A, Serrano J, Gonzalo H, Boada J, Prat J, Portero-Otin M, Pamplona R (2012) Cellular dysfunction in diabetes as maladaptive response to mitochondrial oxidative stress. *Exp Diabetes Res* 2012:696215
- Navia BA, Dafni U, Simpson D, Tucker T, Singer E, McArthur JC, Yiannoutsos C, Zaborski L, Lipton SA (1998) A phase I/II trial of nimodipine for HIV-related neurologic complications. *Neurology* 51(1):221–228
- Nemni R, Sanvito L, Quattrini A, Santuccio G, Camerlingo M, Canal N (2003) Peripheral neuropathy in hepatitis C virus infection with and without cryoglobulinaemia. *J Neurol Neurosurg Psychiatry* 74(9):1267–1271
- Newlands ES (1978) The current role of cancer chemotherapy. *Br J Radiol* 51(610):756–770
- Okada K, Tanaka H, Temporin K, Okamoto M, Kuroda Y, Moritomo H, Murase T, Yoshikawa H (2010) Methylcobalamin increases Erk1/2 and Akt activities through the methylation cycle and promotes nerve regeneration in a rat sciatic nerve injury model. *Exp Neurol* 222(2):191–203
- Osterloh JM, Yang J, Rooney TM, Fox AN, Adalbert R, Powell EH, Sheehan AE, Avery MA, Hackett R, Logan MA, MacDonald JM, Ziegenfuss JS, Milde S, Hou YJ, Nathan C, Ding A, Brown RH Jr, Conforti L, Coleman M, Tessier-Lavigne M, Zuchner S, Freeman MR (2012) dSarm/Sarm1 is required for activation of an injury-induced axon death pathway. *Science* 337(6093):481–484
- Osuntokun BO (1986) Epidemiology of peripheral neuropathies. In: *Neurology*. Springer, Berlin/Heidelberg
- Ozturk E, Arslan AKK, Yerer MB, Bishayee A (2017) Resveratrol and diabetes: a critical review of clinical studies. *Biomed Pharmacother* 95:230–234
- Palencia G, Hernández-Pedro N, Saavedra-Perez D, Peña-Curiel O, Ortiz-Plata A, Ordoñez G, Flores-Estrada D, Sotelo J, Arrieta O (2014) Retinoic acid reduces solvent-induced neuropathy and promotes neural regeneration in mice. *J Neurosci Res* 92(8):1062–1070
- Palumbo PJ, Elveback LR, Whisnant JP (1978) Neurologic complications of diabetes mellitus: transient ischemic attack, stroke, and peripheral neuropathy. *Adv Neurol* 19:593–601
- Pan H-C, Wu H-T, Cheng F-C, Chen C-H, Sheu M-L, Chen C-J (2009) Potentiation of angiogenesis and regeneration by G-CSF after sciatic nerve crush injury. *Biochem Biophys Res Commun* 382(1):177–182
- Pan HC, Sheu ML, Su HL, Chen YJ, Chen CJ, Yang DY, Chiu WT, Cheng FC (2011) Magnesium supplement promotes sciatic nerve regeneration and down-regulates inflammatory response. *Magn Res* 24(2):54–70
- Polydefkis M, Hauer P, Sheth S, Sirdofsky M, Griffin JW, McArthur JC (2004) The time course of epidermal nerve fibre regeneration: studies in normal controls and in people with diabetes, with and without neuropathy. *Brain* 127(Pt 7):1606–1615
- Polydefkis M, Sirdofsky M, Hauer P, Petty BG, Murinson B, McArthur JC (2006) Factors influencing nerve regeneration in a trial of timcodar dimesylate. *Neurology* 66(2):259–261
- Prausnitz MR, Langer R (2008) Transdermal drug delivery. *Nat Biotechnol* 26(11):1261–1268
- Prow TW, Chen X, Prow NA, Fernando GJP, Tan CSE, Raphael AP, Chang D, Ruutu MP, Jenkins DWK, Pyke A, Crichton ML, Raphaelli K, Goh LYH, Frazer IH, Roberts MS, Gardner J, Khromykh AA, Suhrbier A, Hall RA, Kendall MAF (2010) Nanopatch-targeted skin vaccination against West Nile virus and chikungunya virus in mice. *Small* 6(16):1776–1784

- Pyriochou A, Zhou Z, Koika V, Petrou C, Cordopatis P, Sessa WC, Papapetropoulos A (2007) The phosphodiesterase 5 inhibitor sildenafil stimulates angiogenesis through a protein kinase G/ MAPK pathway. *J Cell Physiol* 211(1):197–204
- Rajan B, Polydefkis M, Hauer P, Griffin JW, McArthur JC (2003) Epidermal reinnervation after intracutaneous axotomy in man. *J Comp Neurol* 457(1):24–36
- Rauskolb S, Dombert B, Sendtner M (2017) Insulin-like growth factor 1 in diabetic neuropathy and amyotrophic lateral sclerosis. *Neurobiol Dis* 97:103–113
- Ribeiro CM, Vasconcelos BC, Silva Neto JC, Silva Junior VA, Figueiredo NG (2008) Histopathological analysis of gangliosides use in peripheral nerve regeneration after axonotmesis in rats. *Acta Cir Bras* 23(4):364–371
- Rotshenker S (2011) Wallerian degeneration: the innate-immune response to traumatic nerve injury. *J Neuroinflammation* 8:109–109
- Sahenk Z, Barohn R, New P, Mendell JR (1994) Taxol neuropathy. Electrodiagnostic and sural nerve biopsy findings. *Arch Neurol* 51(7):726–729
- Santoro L, Manganelli F, Briani C, Giannini F, Benedetti L, Vitelli E, Mazzeo A, Beghi E, HCV Peripheral Nerve Study Group, Bassi F, Bibbò G, Cavaletto L, Chemello L, Cimino L, Bogliun G, Faggi LM, Ghiglione E, Iodice R, Girlanda P, Schenone A, Vita G, Zara G (2006) Prevalence and characteristics of peripheral neuropathy in hepatitis c virus population. *J Neurol Neurosurg Psychiatry* 77(5):626–629
- Sharma N, Marzo SJ, Jones KJ, Foecking EM (2010) Electrical stimulation and testosterone differentially enhance expression of regeneration-associated genes. *Exp Neurol* 223(1):183–191
- Shen Q, Pierce JD (2015) Supplementation of coenzyme Q10 among patients with type 2 diabetes mellitus. *Healthcare* 3(2):296–309
- Shi TJ, Zhang MD, Zeberg H, Nilsson J, Grunler J, Liu SX, Xiang Q, Persson J, Fried KJ, Catrina SB, Watanabe M, Arhem P, Brismar K, Hokfelt TG (2013) Coenzyme Q10 prevents peripheral neuropathy and attenuates neuron loss in the db/db- mouse, a type 2 diabetes model. *Proc Natl Acad Sci U S A* 110(2):690–695
- Sima AAF, Zhang W, Xu G, Sugimoto K, Guberski D, Yorek MA (2000) A comparison of diabetic polyneuropathy in type II diabetic BBZDR/Wor rats and in type I diabetic BB/Wor rats. *Diabetologia* 43(6):786–793
- Simonato M, Bennett J, Boulis NM, Castro MG, Fink DJ, Goins WF, Gray SJ, Lowenstein PR, Vandenbergh LH, Wilson TJ, Wolfe JH, Glorioso JC (2013) Progress in gene therapy for neurological disorders. *Nat Rev Neurol* 9(5):277–291
- Sinnreich M, Taylor BV, Dyck PJB (2005) Diabetic neuropathies: classification, clinical features, and pathophysiological basis. *Neurologist* 11(2):63–79
- Smyth K, Affandi JS, McArthur JC, Bowtell-harris C, Mijch AM, Watson K, Costello K, Woolley IJ, Price P, Wesselingh SL, Cherry CL (2007) Prevalence of and risk factors for HIV-associated neuropathy in Melbourne, Australia 1993–2006. *HIV Med* 8(6):367–373
- Snyder MJ, Gibbs LM, Lindsay TJ (2016) Treating painful diabetic peripheral neuropathy: an update. *Am Fam Physician* 94(3):227–234
- Sobeski JK, Kerns JM, Safanda JF, Shott S, Gonzalez MH (2001) Functional and structural effects of GM-1 ganglioside treatment on peripheral nerve grafting in the rat. *Microsurgery* 21(3):108–115
- Sperry RW (1963) Chemoaffinity in the orderly growth of nerve fiber patterns and connection. *Proc Natl Acad Sci U S A* 50:703–710
- Suarez-Mendez S, Tovilla-Zárate CA, Juárez-Rojop IE, Bermúdez-Ocaña DY (2018) Erythropoietin: a potential drug in the management of diabetic neuropathy. *Biomed Pharmacother* 105:956–961
- Summers DW, DiAntonio A, Milbrandt J (2014) Mitochondrial dysfunction induces Sarm1-dependent cell death in sensory neurons. *J Neurosci* 34(28):9338–9350
- Sun R, Zhang J, Wei H, Meng X, Ding Q, Sun F, Cao M, Yin L, Pu Y (2017) Acetyl-L-carnitine partially prevents benzene-induced hematotoxicity and oxidative stress in C3H/He mice. *Environ Toxicol Pharmacol* 51:108–113

- Sunderland S (1947) Rate of regeneration in human peripheral nerves; analysis of the interval between injury and onset of recovery. *Arch Neurol Psychiatr* 58(3):251–295
- Surmelioglu O, Sencar L, Ozdemir S, Tarkan O, Dagkiran M, Surmelioglu N, Tuncer U, Polat S (2017) Effects of agmatine sulphate on facial nerve injuries. *J Laryngol Otol* 131(3):221–226
- Ta LE, Espeset L, Podratz J, Windebank AJ (2006) Neurotoxicity of oxaliplatin and cisplatin for dorsal root ganglion neurons correlates with platinum–DNA binding. *Neurotoxicology* 27(6):992–1002
- Takagi T, Nakamura M, Yamada M, Hikishima K, Momoshima S, Fujiyoshi K, Shibata S, Okano HJ, Toyama Y, Okano H (2009) Visualization of peripheral nerve degeneration and regeneration: monitoring with diffusion tensor tractography. *NeuroImage* 44(3):884–892
- Tanaka S, Avigad G, Brodsky B, Eikenberry EF (1988) Glycation induces expansion of the molecular packing of collagen. *J Mol Biol* 203(2):495–505
- Tardiolo G, Bramanti P, Mazzon E (2018) Overview on the effects of N-Acetylcysteine in neurodegenerative diseases. *Molecules* 23(12):3305
- Terenghi G (1999) Peripheral nerve regeneration and neurotrophic factors. *J Anat* 194(1):1–14
- Theiss C, Meller K (2000) Taxol impairs anterograde axonal transport of microinjected horseradish peroxidase in dorsal root ganglia neurons in vitro. *Cell Tissue Res* 299(2):213–224
- Toftthagen C (2010) Patient perceptions associated with chemotherapy-induced peripheral neuropathy. *Clin J Oncol Nurs* 14(3):E22–E28
- Tordjman S, Chokron S, Delorme R, Charrier A, Bellissant E, Jaafari N, Fougere C (2017) Melatonin: pharmacology, functions and therapeutic benefits. *Curr Neuropharmacol* 15(3):434–443
- Turkiew E, Falconer D, Reed N, Hoke A (2017) Deletion of *Sarm1* gene is neuroprotective in two models of peripheral neuropathy. *J Peripher Nerv Syst* 22(3):162–171
- Vlassara H, Brownlee M, Cerami A (1981) Nonenzymatic glycosylation of peripheral nerve protein in diabetes mellitus. *Proc Natl Acad Sci U S A* 78(8):5190–5192
- Wang MS, Davis AA, Culver DG, Glass JD (2002) WldS mice are resistant to paclitaxel (taxol) neuropathy. *Ann Neurol* 52(4):442–447
- Wang L, Chopp M, Szalad A, Jia L, Lu X, Lu M, Zhang L, Zhang Y, Zhang R, Zhang ZG (2015a) Sildenafil ameliorates long term peripheral neuropathy in type II diabetic mice. *PLoS One* 10(2):e0118134
- Wang L, Yuan D, Zhang D, Zhang W, Liu C, Cheng H, Song Y, Tan Q (2015b) Ginsenoside re promotes nerve regeneration by facilitating the proliferation, differentiation and migration of Schwann cells via the ERK- and JNK-dependent pathway in rat model of sciatic nerve crush injury. *Cell Mol Neurobiol* 35(6):827–840
- Wang J, Qiao Y, Yang R-S, Zhang C-K, Wu H-H, Lin J-J, Zhang T, Chen T, Li Y-Q, Dong Y-L, Li J-L (2017) The synergistic effect of treatment with triptolide and MK-801 in the rat neuropathic pain model. *Mol Pain* 13:1744806917746564
- Wesselink E, Winkels RM, van Baar H, Geijsen AJMR, van Zutphen M, van Halteren HK, Hansson BME, Radema SA, de Wilt JHW, Kampman E, Kok DEG (2018) Dietary intake of magnesium or calcium and chemotherapy-induced peripheral neuropathy in colorectal cancer patients. *Nutrients* 10(4):398
- WHO (2011) Global status report on noncommunicable diseases 2010: description of the global burden of NCDs, their risk factors and determinants. WHO, Geneva
- Winer JB (2014) An update in Guillain-Barré syndrome. *J Autoimmune Dis* 2014:793024
- Won JC, Kim SS, Ko KS, Cha BY (2014) Current status of diabetic peripheral neuropathy in Korea: report of a hospital-based study of type 2 diabetic patients in Korea by the diabetic neuropathy study group of the Korean diabetes association. *Diabetes Metab J* 38(1):25–31
- Wright JC (1984) Cancer chemotherapy: past, present, and future – part I. *J Natl Med Assoc* 76(8):773–784
- Wu Z, Mata M, Fink DJ (2012) Prolonged regulatable expression of EPO from an HSV vector using the LAP2 promoter element. *Gene Ther* 19(11):1107–1113

- Xiong Y, Mahmood A, Chopp M (2010) Angiogenesis, neurogenesis and brain recovery of function following injury. *Curr Opin Invest Drugs* 11(3):298–308
- Xu Q, Pan J, Yu J, Liu X, Liu L, Zuo X, Wu P, Deng H, Zhang J, Ji A (2013) Meta-analysis of methylcobalamin alone and in combination with lipoic acid in patients with diabetic peripheral neuropathy. *Diabetes Res Clin Pract* 101(2):99–105
- Yamamoto K, Amako M, Yamamoto Y, Tsuchihara T, Nukada H, Yoshihara Y, Arino H, Fujita M, Uenoyama M, Tachibana S (2013) Therapeutic effect of exendin-4, a long-acting analogue of glucagon-like peptide-1 receptor agonist, on nerve regeneration after the crush nerve injury. *Biomed Res Int* 2013:315848
- Yamasaki T, Fujiwara H, Oda R, Mikami Y, Ikeda T, Nagae M, Shirai T, Morisaki S, Ikoma K, Masugi-Tokita M, Yamada K, Kawata M, Kubo T (2015) In vivo evaluation of rabbit sciatic nerve regeneration with diffusion tensor imaging (DTI): correlations with histology and behavior. *Magn Reson Imaging* 33(1):95–101
- Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, Pinsky D, Stern D (1994) Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J Biol Chem* 269(13):9889–9897
- Yang Y, Zhang YG, Lin GA, Xie HQ, Pan HT, Huang BQ, Liu JD, Liu H, Zhang N, Li L, Chen JH (2014) Spinal changes of a newly isolated neuropeptide endomorphin-2 concomitant with vincristine-induced allodynia. *PLoS One* 9(2):e89583
- Yin Z-S, Zhang H, Gao W (2010) Erythropoietin promotes functional recovery and enhances nerve regeneration after peripheral nerve injury in rats. *AJNR Am J Neuroradiol* 31(3):509–515
- Yorek MA, Dunlap JA, Ginsberg BH (1987) myo-Inositol metabolism in 41A3 neuroblastoma cells: effects of high glucose and sorbitol levels. *J Neurochem* 48(1):53–61
- Yuki N, Yamada M, Koga M, Odaka M, Susuki K, Tagawa Y, Ueda S, Kasama T, Ohnishi A, Hayashi S, Takahashi H, Kamijo M, Hirata K (2001) Animal model of axonal Guillain-Barré syndrome induced by sensitization with GM1 ganglioside. *Ann Neurol* 49(6):712–720
- Zan Y, Kuai CX, Qiu ZX, Huang F (2017) Berberine ameliorates diabetic neuropathy: TRPV1 modulation by PKC pathway. *Am J Chin Med* 45(8):1709–1723
- Zanon R, Cartarozzi L, Victório S, Moraes J, Morari J, Velloso L, Oliveira A (2010) Interferon (IFN) beta treatment induces major histocompatibility complex (MHC) class I expression in the spinal cord and enhances axonal growth and motor function recovery following sciatic nerve crush in mice. *Neuropathol Appl Neurobiol* 36(6):515–534
- Zhang Z, Liew CW, Handy DE, Zhang Y, Leopold JA, Hu J, Guo L, Kulkarni RN, Loscalzo J, Stanton RC (2010) High glucose inhibits glucose-6-phosphate dehydrogenase, leading to increased oxidative stress and beta-cell apoptosis. *FASEB J* 24(5):1497–1505
- Zhang X, Zhang X, Wang F (2012) Intracellular transduction and potential of Tat PTD and its analogs: from basic drug delivery mechanism to application. *Expert Opin Drug Deliv* 9(4):457–472
- Zhang L, Mao W, Guo X, Wu Y, Li C, Lu Z, Su G, Li X, Liu Z, Guo R, Jie X, Wen Z, Liu X (2013) *Ginkgo biloba* extract for patients with early diabetic nephropathy: a systematic review. *Evid Based Complement Alternat Med* 2013:689142
- Zhang YG, Sheng QS, Wang HK, Lv L, Zhang J, Chen JM, Xu H (2014) Triptolide improves nerve regeneration and functional recovery following crush injury to rat sciatic nerve. *Neurosci Lett* 561:198–202
- Zhang H-N, Sun Y-J, He H-Q, Li H-Y, Xue Q-L, Liu Z-M, Xu G-M, Dong L-H (2018a) Berberine promotes nerve regeneration through IGFR-mediated JNK-AKT signal pathway. *Mol Med Rep* 18(6):5030–5036
- Zhang Q, Ji L, Zheng H, Li Q, Xiong Q, Sun W, Zhu X, Li Y, Lu B, Liu X, Zhang S (2018b) Low serum phosphate and magnesium levels are associated with peripheral neuropathy in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract* 146:1–7
- Zheng X, Ouyang H, Liu S, Mata M, Fink DJ, Hao S (2011) TNFalpha is involved in neuropathic pain induced by nucleoside reverse transcriptase inhibitor in rats. *Brain Behav Immun* 25(8):1668–1676

- Zheng L, Hui Q, Tang L, Zheng L, Jin Z, Yu B, Wang Z, Lin P, Yu W, Li H, Li X, Wang X (2015) TAT-mediated acidic fibroblast growth factor delivery to the dermis improves wound healing of deep skin tissue in rat. *PLoS One* 10(8):e0135291
- Zhou Z, Peng X, Hao S, Fink DJ, Mata M (2008) HSV-mediated transfer of interleukin-10 reduces inflammatory pain through modulation of membrane tumor necrosis factor alpha in spinal cord microglia. *Gene Ther* 15(3):183–190
- Zhou Y, Tao J, Yu H, Ni J, Zeng L, Teng Q, Kim KS, Zhao GP, Guo X, Yao Y (2012) Hcp family proteins secreted via the type VI secretion system coordinately regulate *Escherichia coli* K1 interaction with human brain microvascular endothelial cells. *Infect Immun* 80(3):1243–1251
- Zhou P, Xie W, He S, Sun Y, Meng X, Sun G, Sun X (2019) Ginsenoside Rb1 as an anti-diabetic agent and its underlying mechanism analysis. *Cell* 8(3):204
- Zhu Z, Zhou X, He B, Dai T, Zheng C, Yang C, Zhu S, Zhu J, Zhu Q, Liu X (2015) *Ginkgo biloba* extract (EGb 761) promotes peripheral nerve regeneration and neovascularization after acellular nerve allografts in a rat model. *Cell Mol Neurobiol* 35(2):273–282
- Ziaei S, Halaby R (2016) Immunosuppressive, anti-inflammatory and anti-cancer properties of triptolide: a mini review. *Avicenna J Phytomed* 6(2):149–164
- Ziegler D, Gries FA, Spüler M, Lessmann F (1992) The epidemiology of diabetic neuropathy. *J Diabetes Complicat* 6(1):49–57
- Zochodne DW, Levy D (2005) Nitric oxide in damage, disease and repair of the peripheral nervous system. *Cell Mol Biol (Noisy-le-Grand)* 51(3):255–267



Rehabilitation of Nerve Injuries

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Abstract

Historically, surgical outcomes following peripheral nerve injury (PNI) have focused on the motor (movement and strength) and sensory losses that patients experience. Rehabilitation following nerve injury goes well beyond these empirical elements of function. Therapists recognize that functional loss resulting from a PNI is not contained to the physical manifestation of symptoms but also has a wider impact on personal and societal interactions. These patient-specific factors have been highlighted in many qualitative research studies. Understanding a patient’s experiences of recovery provides insight into what reinnervation and the resultant return of movement, muscle strength, and sensation mean to the individual. This chapter outlines factors which should be considered when constructing an individual rehabilitation program. Examples are primarily drawn from injuries to the upper extremity, but the principles of rehabilitation can be applied to most peripheral nerve injuries.

1 Introduction

Injuries to peripheral nerves can have a significant impact on an individual’s level of function (Khan and Birch 2001). Depending upon the mechanism, severity and location of the injury this can range from moderate and short term to life changing and permanent. Furthermore, the extent of functional loss is also dictated by the proximity of the injury to the spine and the brachial plexus. This, in turn, influences recovery times, with more proximal injuries having further to regrow. The rate of axonal growth is estimated to be 1 mm/day (Gutmann et al. 1942; Seddon et al. 1943; Trojaborg 1970). This time aspect is pertinent because the possibility of muscle function recovery is reduced the longer the time to reinnervation (Fu and Gordon 1995). Intramuscular changes occur which can render the muscle anatomically and physiologically incapable of contraction (Wu et al. 2014). Furthermore, cortical reorganization is needed to allow integration into functional movement patterns (Rosén 1996). In severe nerve injuries, surgery can improve functional outcome and assist in attaining rehabilitation targets. A previous chapter has outlined the surgical interventions which may include decompression and direct repair or nerve grafting to restore or reconstruct the damaged nerves’ functions. If nerve surgery is deemed necessary, but the delay to treatment has been significant (or if the injury location is a great distance from the target muscles), nerve transfer surgery can be considered. This surgical technique redirects a functioning nerve into the denervated muscle in order to reinnervate it (see chapter ► “Surgical Techniques in Nerve Repair” of this book). In this situation a different postoperative rehabilitation

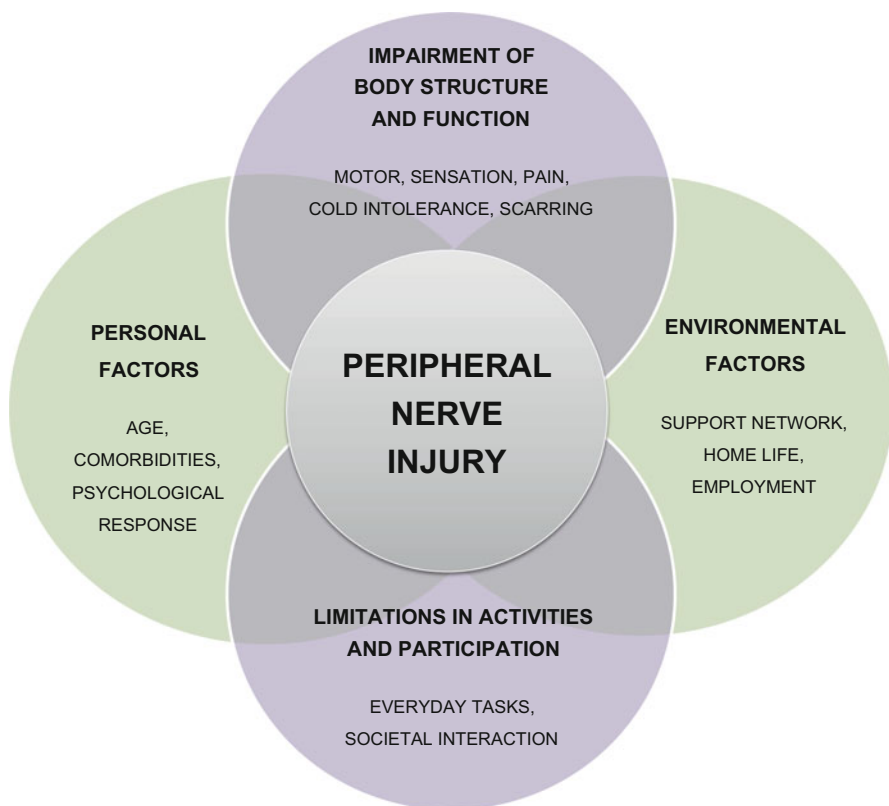


Fig. 1 Adapted conceptualization of the WHO-ICF (2001) representing the interaction and effect of various factors on recovery following PNI

program is required to that if the native nerve reinnervates its original muscle (see Sects. 4.2.3 and 4.2.4 below).

The World Health Organisation International Classification of Functioning, Disability and Health (WHO-ICF) is a framework for measuring health and disability at both an individual and population level (WHO 2001). The framework epitomizes a bio-psycho-social approach, whereby individual impairments and the person's perceptions of their function are considered alongside their ability to participate in everyday activities (Fig. 1). In addition, the framework recognizes that personal factors (such as psychological well-being), environmental factors (such as the ability to access the workplace), and biological factors (such as age) will affect their current state and future outcome. The WHO-ICF informs the structure of this chapter.

Rehabilitation after a peripheral nerve injury follows certain principles including: maintenance of passive range within affected joints, minimizing cortical changes, sensory re-training, pain management, progressive strengthening (as recovery allows), and optimizing functional use of the limb. However, within these principles

treatment should always be personalized and tailored to the individual. In order to achieve this, therapists must gain an understanding of the underlying personal and environmental factors which surround the patient and may influence their ability to engage in rehabilitation.

2 Personal Factors

2.1 Biological Factors

Biological factors limiting recovery have been well documented within many health conditions. Factors which typically hinder recovery include: smoking, diabetes mellitus, obesity, and advancing age. The literature on the prognostic value of the aforementioned factors following PNI is limited. The delay from injury to surgery and high body mass index have been reported as factors that affect the outcome of nerve surgery (Lee et al. 2012). Aging is known to affect the functional and electrophysiologic properties of the peripheral nervous system and is the most widely documented factor which can limit recovery (Hundepool et al. 2015; Lee et al. 2012; Verdú et al. 2000).

The relationship between smoking and functional outcomes has been investigated within animal models. Exposure to cigarette smoke has been shown to adversely affect healing, and is associated with a slower functional recovery following PNI (Pankow et al. 1974; al-Ghazal et al. 1994; Rinker et al. 2011). In a study on human PNI patients, smoking was not found to be a prognostic indicator of recovery (Hundepool et al. 2015).

2.2 Psychosocial Factors

PNI and particularly brachial plexus injuries (BPI) can be life changing and cause psychological impairments. Body image, self-esteem, and hope/expectations are all key issues that have been highlighted during qualitative studies (McDonald and Pettigrew 2014; Wellington 2010; Brown et al. 2018). Other symptoms such as flashbacks, nightmares, feelings of sadness, and hopelessness have also been reported to be prevalent (Chemnitz et al. 2013). A systematic review by Miller et al. (2017) identified that post-traumatic stress disorder and clinical depression were common consequences of upper limb PNI. Quality of life (QoL) is therefore significantly reduced following PNI (Wellington 2010; Gray 2016; Ashwood et al. 2017; Verma et al. 2019).

Patient reported outcome measures (PROs) provide insights into the individuals' assessment of their function and assist with the direction of care. Measures of QoL which have been utilized within the clinical environment include the 36-item Short Form Health Survey (SF-36) and EuroQoL 5D (EQ 5D) (Dy et al. 2015). However, it must be recognized that although PROs can offer some

information with regard to how an individual is living with an injury, they are restricted in the level of information that they provide. Individual goal setting is essential as it will ensure that treatment plans address the individual's identified issues with QoL.

Identification of psychological impairments and referral to healthcare professionals who can assist with the development of coping mechanisms and acceptance is vital in order to optimize recovery and long-term clinical outcomes. Furthermore, to fully engage individuals in the rehabilitation process, it is important to gain an insight into their level of understanding and acceptance of their injury. It has been postulated that the somewhat unknown prognosis following BPI can incite longer time scales of acceptance (Davidson 2004). Patient education (regarding the injury that they have suffered, the expected time to recovery, and the rehabilitation process) is essential to achieve the best possible outcome for the individual (Janssen et al. 2019). The patient's personality type and preferred learning styles should also be considered when tailoring the treatment approach and dispensing information for the patient to refer to once they are within their home environment (Moorhead et al. 2011; Vikström et al. 2018).

3 Environmental Factors

Feelings of social isolation are common during recovery following PNI (Verma et al. 2019; Chemnitz et al. 2013). The importance of a support network is acknowledged (McDonald and Pettigrew 2014). In the case of severe injuries, the support of fellow patients and support groups are often sought (Janssen et al. 2019). The Traumatic Brachial Plexus Injury group (www.tbpi-group.org/) and United Brachial Plexus Network (www.ubpn.org/) are two networks that have a vital role in the rehabilitation of many individuals.

Motivation is a central component to engagement in therapeutic regimes, with an individual only fully engaged in the process if treatment is tailored to their needs and life roles (Brawley and Culos-Reed 2000). These will vary according to employment and relationship status (Wellington 2010). Return to work assessments should consider both the physical and psychological aspects of their role including: travel and access to the workplace, activity pacing, and adaptations to vehicles and equipment.

4 Body Structure and Function

Physical assessment of the patient should be conducted at regular intervals in order to identify early reinnervation, monitor the rehabilitation progress, and adjust an individual's rehabilitation program according to assessment findings. These objective measures will aid subsequent decision making regarding the early and late treatment options that may optimize function.

4.1 Motor

In clinical practice the recovery of motor function after a nerve injury is commonly assessed using objective measures such as the Medical Research Council (MRC) muscle grading system (Table 1) (Medical Research Council 1943; Dyck et al. 2005).

This system ranges from grade 0 (no contraction) to 5 (normal) and is recognized as a quick and easy method to evaluate muscle activity. However, the system has been criticized particularly in terms of the 4/5 grade (MacAvoy and Green 2007). Grade 4 on the scale is defined as “active movement against gravity and resistance” but the grade lacks sensitivity. For example, in the case of ankle plantarflexion both the ability to resist minimal resistance and movement of body weight during a calf raise would be classified as a grade 4.

MacAvoy and Green (2007) reported that the grade 4 has a disproportionate share of the entire grading scale, with 96% of muscle strength assessments being able to be categorized as a grade 4/5. The use of manual muscle testing therefore risks missing critical changes of motor recovery because of its lack of sensitivity. The use of qualifiers (4−, 4+, 5−) for grades 4 and 5 has been implemented in an attempt to address these issues (Kendall and McCreary 1983). However, these are still subjectively decided by the tester and therefore open to interpretation.

More recently force transducers or dynamometers have been used in an attempt to evaluate muscle torque and fatigability after nerve injury (Bertelli and Ghizoni 2014; Quick et al. 2016; Wilcox et al. 2019). Hand-held dynamometers (Fig. 2a) are being favored within clinics and research due to their ability to provide continuous data, their portability and ease of use. In addition, the immediate feedback that they provide is beneficial to the patient and therapist. Hand grip (Fig. 2b) and thumb pinch dynamometers (Fig. 2c) are also increasingly being used to evaluate strength and function following upper limb nerve injury. These measures provide information on the combined actions of the muscles in the hand and are thought to reflect the functional changes experienced by the individual. In order to achieve good reliability, the same positioning and parameters should be applied when serially assessing patients (McDermid et al. 2015).

Table 1 Medical Research Council (MRC) muscle grading system (Medical Research Council 1943)

Grade (/5)	Description
0	No contraction
1	Flicker or trace of contraction
2	Full range of motion with gravity eliminated
3	Active movement against gravity
4	Active movement against gravity and resistance
5	Normal power across full range of movement

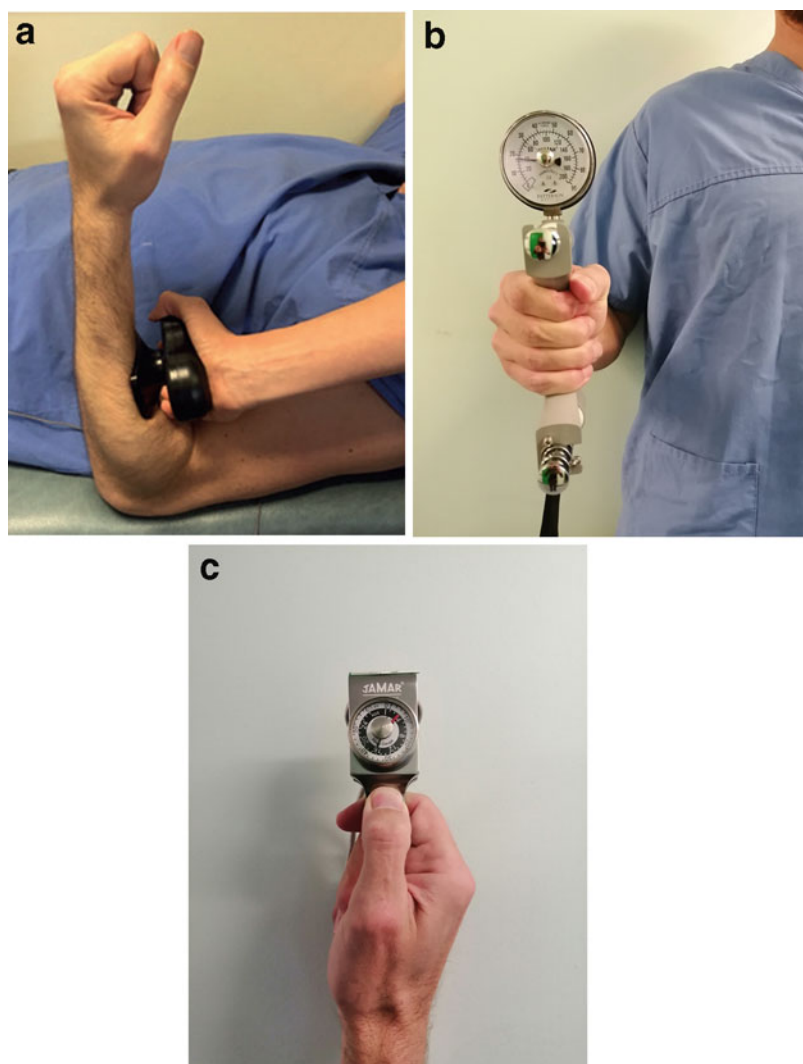


Fig. 2 Commonly used force transducers. (a) Hand-held dynamometer, (b) hand grip dynamometer, and (c) pinch grip dynamometer

4.2 Rehabilitation of the Motor System Following Peripheral Nerve Surgery

After a nerve injury there are measurable functional and structural changes to the peripheral and the central nervous system which continue through reinnervation and reconstructive surgery (Goswami et al. 2016; Chen et al. 2003; Anastakis et al. 2008; Merzenich and Jenkins 1993). The number of motor axons and the degree of muscle

innervation are important factors influencing muscle strength. However, the importance of cortical changes cannot be underestimated; to optimize outcomes cortical reorganization training is essential (see Sect. 4.3.1 below).

4.2.1 Preoperative

If the patient with a peripheral nerve injury is seen preoperatively, baseline measures of range of movement and strength should be obtained. This will help with monitoring postoperative recovery. Donor muscle strengthening exercises should be taught if the patient is having a nerve transfer procedure. Nerve transfers will create an altered neural pathway and rehabilitation must focus on reinforcing these new pathways (Kahn and Moore 2016; Novak 2008; Hill et al. 2019). The movement pattern the surgery is aiming to improve should be practiced prior to surgery on the contralateral limb. The aim is to increase a patient's understanding of their surgery and the concept of a "donor" nerve and a "receiving" muscle. For example, if the intended surgery is a transfer of a radial nerve branch from one of the triceps muscle bellies to the deltoid muscle, the patient is asked to actively extend the contralateral elbow while simultaneously abducting the contralateral shoulder. This can also be completed using a mirror giving the impression that the affected limb is completing the movement with the underlying aim of activating mirror neurons and developing new motor pathways (Gallese et al. 1996; Carroll et al. 2006).

Joint passive range of movement needs to be maintained as joint stiffness will negate the potential success of nerve surgery. In addition, muscle length may change because of the unopposed force of the antagonist muscle, improper positioning and the weight of the limb itself. A muscle that is overly stretched or shortened may have difficulty generating force once it begins to regain function (Gordan et al. 1966).

4.2.2 Postoperative Protective Phase

It is important for the therapist to see the patient on the first postoperative visit for advice and education. The operation notes need to be carefully reviewed.

Whether the surgery involves a direct repair, graft, conduit, or a nerve transfer the priority is to protect the nerve coaptation and minimize pain. Depending on the area of surgery a sling, orthosis, or custom-made splint may be used for protection of the surgical site. A minimum period of immobilization following nerve surgery is recommended to limit adherence of the nerve to the surrounding soft tissues. Recommendations vary from 7 to 21 days (Kahn and Moore 2016; Novak 2008). Novak and von der Heyde (2015) recommend that nerve transfers and grafts are immobilized for shorter periods of time (7–10 days) as the surgery is performed at laxity. However, if other soft tissues are repaired (e.g., tendon repairs) at the same time as the nerve surgery, the rehabilitation will follow the tendon repair guidance. At all times close communication with the surgeon and in-depth knowledge of the surgery performed will guide timing and intensity of rehabilitation.

4.2.3 Post Protection

One of the greatest challenges for a patient undergoing any nerve surgery is waiting for a functional outcome. Patients may get depressed, anxious, and frustrated when

no motor recovery is achieved for months. It is essential that the expectations of recovery time are explained preoperatively. This may need to be reiterated many times.

Following nerve transfer surgery the first observation of contraction is usually seen around 4–6 months following an Oberlin transfer and 6–9 months following a Somsak nerve transfer (Hill et al. 2019). However, definite timescales are difficult to predict and many factors influence the rate and quality of the reinnervation.

Once the early postoperative protective phase is over, exercises can be commenced to prevent joint stiffness and to allow neural gliding at the site of surgery.

Following a nerve transfer there may be weakness and atrophy in the donor muscle postoperatively. For example, following a transfer of the medial triceps branch to the axillary nerve, motor function in the medial head of triceps is lost. Functional recovery needs to be preserved through strengthening of the remaining innervated triceps heads.

During early postoperative nerve transfer rehabilitation (before a muscle twitch is observed in the newly innervated muscle), exercises of the donor muscle are recommended. Rehabilitation can begin with resisted donor muscle contractions with concurrent passive- or active-assisted repetition of the anticipated movement. For example, a nerve transfer that aims to reinnervate the elbow flexors (biceps and brachialis muscles) using fascicles (supplying the wrist flexors) from the ulnar and/or median nerves: While waiting for reinnervation of the elbow flexors, repeated *passive* elbow flexion with simultaneous *active* wrist flexion reinforces the altered neural pathway for the recipient muscle (biceps and brachialis). This can be done with a weighted bar held with both hands. This bilateral movement assists in the learning of new motor patterns, providing feedback and cortical input of the new anticipated movement from the contralateral side (Kahn and Moore 2016).

4.2.4 Muscle Activation

When a twitch (1/5 MRC) is perceived in the newly innervated muscle the focus of therapy changes to gravity reduced or even gravity assisted exercises; this aims to activate the muscle. Place and hold exercises can initially be used. These involve using the unaffected arm to place the affected joint into a mid-range position. The mid-range position is used as the length tension relationship in this position facilitates easier recruitment of the weak and newly innervated muscle. The support of the unaffected arm is gradually reduced while the patient actively (isometrically) maintains the position. The exercise can be progressed to different ranges of motion. Taking again the example of a nerve transfer to reanimate elbow flexion where nerve fascicles have been transferred from the median and ulnar nerve (i.e., elbow flexion is produced by contraction of the wrist flexors; essentially becoming a combined movement). To facilitate relearning of elbow flexion, the elbow is placed in mid-range flexion (90°) and the patient is asked to then contract wrist flexors to initiate the activity of the newly innervated elbow flexors. Initially this exercise can be carried out in a supine position where the gravitational force on the elbow flexors is reduced (2/5 MRC). As the muscle(s) strengthen, patient positioning can be progressed toward sitting and standing to increase the effect of gravity on the biceps and

brachialis muscles (3/5 and above MRC). Bilateral movements are again encouraged to provide cortical input from the uninjured extremity (Hill et al. 2019; Kahn and Moore 2016; Novak 2008; Anastakis et al. 2008).

There is evidence that newly innervated muscles fatigue quickly (Wilcox et al. 2019) and this symptom has been reported by patients (Brown et al. 2018). It is therefore important for the duration and intensity of exercise sessions to be adapted in consideration of this; shorter sessions are recommended (Novak and von der Heyde 2015). High repetition and low resistance are thought to maximize neural input with repetition, while limiting fatigue, but this has yet to be proven in clinical studies. However, it is important to remember that the extent and stage of muscle reinnervation will determine exercise prescription. Therefore regimens should be tailored to the individual and altered over time in response to clinical assessment findings.

In nerve transfer rehabilitation the separation of the donor muscle activity from the newly innervated muscle function will take time (and in some patients the need to perform the combined motions persists). Resistance exercises using hand-held weights or elastic bands can be started once greater than 3/5 MRC muscle strength is achieved. Once motor recovery allows, functional tasks and personalized rehabilitation should be commenced and directed toward each patients' goals. Visual, sensory, and emotional cues have all been shown to enhance the receptivity of regions of the brain responsible for motor learning (Masterson 2015). Emotional states in particular have been shown to enhance or inhibit learning and achievement of a goal (Immordino-Yang 2008).

Whether following a nerve transfer or nerve repair it is worth bearing in mind that in most cases there are fewer motor axons innervating the muscle compared to prior to the injury. Therefore, final strength will vary and may take up to 5 years to recover.

4.3 Adjuncts to Rehabilitation

4.3.1 Enhancing Cortical Representation Training

In addition to the progression of exercises in response to the level of muscle activation it is essential that a concomitant cortical reorganization training program is commenced early in the rehabilitation program. The aim of this is to maintain or regain cortical representation of the paralyzed body part while the muscles and sensory pathways reinnervate. This can be begun even when no active muscle contraction is possible. It is important that the amount of time spent doing the exercises is feasible to the patient and that they are easily able to do the exercises without feeling overwhelmed (Sturma et al. 2018). Strategies such as consistently keeping the injured body part within the visual field can be the base line of such approach. This can be progressed to lateralization training whereby the patient uses an app or prepared cards which show the left and right extremities in a random order (Moseley 2012). The patient reviews the images one by one and reports whether a right or left extremity is shown (Moseley 2012; Sturma et al. 2018). An additional strategy involves the patient using their "mind's eye" to imagine movements within

the affected body part. These may include isolated movements (such as imagining elbow flexion alone; in the case of a lesion affecting the musculocutaneous nerve) or functional movements (such as imagining using the arm to bring a glass to drink from using elbow flexion; in the same scenario). The action and the time spent should be determined by the patient's ability to imagine and visualize the chosen movement (Sturma et al. 2018). The use of a mirror or mirror box may also be included into therapy to create the illusion of active movement within the paralyzed limb (Ramachandran and Rogers-Ramachandran 1996; Rothgangel et al. 2011). In this scenario a mirror is placed in the midline, in front of the patient, so that they are able to see their reflection in place of the paralyzed limb (it is important that the patient is unable to see the injured side and that their mind can imagine/be convinced that the image they see is their affected limb). The patient is then to perform a slow, purposeful, movement with the non-affected arm while looking in the mirror. The patient should be instructed to move "both sides" for 5–10 min twice a day. Although the injured side will not move, it is still important to generate the illusion of simultaneous movement of both limbs (Rothgangel et al. 2011; Sturma et al. 2018).

4.3.2 Splinting

The therapist may need to use orthoses or splints for positioning to maintain optimal soft tissue length and protect the surgical site. It is important when providing splints that patients are educated on: loss of sensation, how to monitor for pressure areas, and how to fit the splints and their duration of wear. Following provision of a splint ongoing review and reassessment is imperative.

4.3.3 Electrical Stimulation

The time delays implicit in axonal regeneration can produce prolonged periods of muscle denervation. During periods of denervation changes occur within the muscle including: interstitial fibrosis, loss of motor end plates, and loss of contractile proteins (Wu et al. 2014). Consequently, longer durations of denervation are associated with poorer outcomes (Fu and Gordon 1995). It therefore makes sense to try to maintain muscle health within this time period by external means. Many studies report favorable results with the application of electrical stimulation (ES) via surface electrodes, to patients who have suffered muscle paralysis secondary to spinal cord injury (SCI) and stroke (Gordon et al. 1990; Neumayer et al. 1997). In these patients, intramuscular ambience has been maintained with reports of muscle bulk and torque being preserved (Kern et al. 2002). However, in both SCI and stroke the peripheral nerve to the muscle is intact with the lesion causing a loss of connection with the brain (i.e., the muscle is not denervated but decorticated). The pathology of PNI is quite different to those of the CNS. Muscle contraction occurs through a loop which requires the lower motor neurone to be in continuity with the spinal cord.

Animal studies have attempted to show maintenance of muscle health with direct stimulation of denervated muscles via intramuscular electrodes (Al-Majed et al. 2000). However, the translational benefit to human cases has not been established. No improvement in outcome has been associated with the application of galvanic stimulation in humans with denervated muscles.

Functional ES once signs of reinnervation occur may be of benefit during the early stages of reinnervation (once MRC grade 1 and 2 muscle contraction is present). Parameters for use have not been identified (Brown 2018). When ES is utilized it should be integrated within the patients' home exercise program or a functional activity.

4.3.4 Biofeedback

Cortical plasticity and motor relearning play a major role during functional recovery. It has been shown that recovery after surgical nerve reconstruction is both a function of peripheral nerve regeneration and adaptations within the CNS, making use of the brain's plastic capacity (Dahlin et al. 2017). Successful neuromuscular rehabilitation requires detailed afferent feedback. Biofeedback applications measure biological information and feed them back to the patient to increase awareness and control (Neblett 2016). Surface electromyographic biofeedback (sEMG) provides feedback of muscle activity by conversion of myoelectrical activity into visual and/or auditory information (Cram 2003; Giggins et al. 2013; Kim 2017). This can be in the form of color-coded graphs or differing sounds displayed via a device which the patient observes. Once clinical signs of target muscle reinnervation are present (observable or palpable volitional contractions within the muscle – MRC grade 1), sEMG biofeedback can be commenced (Novak 2008; Sturma et al. 2018). The therapeutic use of sEMG can be continued until the patient is voluntarily able to activate and control antagonistic pairs of muscles (Novak and von der Heyde 2013; Sturma et al. 2018).

4.3.5 Hydrotherapy

Hydrotherapy and water-based exercises have long been used by clinicians in order to assist with rehabilitation (Mooventhan and Nivethitha 2014). In the case of PNI and BPI hydrotherapy is often used as an adjunct to therapy. Qualitative studies have identified that it is a modality that patients enjoy and find of great benefit especially within the first stages of rehabilitation when muscle recruitment and control are diminished (Brown et al. 2018).

4.4 Sensory

Sensibility is described as the ability to receive and perceive sensation (Dellon 1991). The neuroanatomy and physiology of touch via the skin are characterized by differing kinds of cutaneous mechanoreceptors (McGlone and Reilly 2010). Information is then projected to the sensory cortex for interpretation. When a nerve injury occurs, recovery of sensation is only partly due to axons crossing the injured site and reaching the cutaneous receptors within the dermis and epidermis. During an insensate period, neuroplasticity leads to alterations of the projection of the body within the somatosensory cortex. Therefore, the ability of the brain to interpret signals from intact sensory receptors is sometimes altered.

Limited sensibility within the hand and upper limb greatly impacts on function, occupation, QoL, and psychological well-being. It is therefore crucial that sensory assessment and re-education is addressed early within PNI rehabilitation programs.

Due to the length of time it can take for a peripheral nerve to recover it is important that instruments used to document progress during rehabilitation are capable of accurately measuring change over time (Fonseca et al. 2018).

Sensibility assessments are divided into four categories: Threshold, Discrimination, Quantification, and Identification (Fess 1995).

1. **Threshold tests:** The first level of testing to assess whether an individual can detect a stimulus or not (Fess 1995). The tests provide information on the ability to detect light touch, deep pressure, and also temperature and pain thresholds. The Semmes-Weinstein monofilaments and the Weinstein Enhanced Sensory Test (WEST) are static touch assessments used to monitor return of sensation in the early stages of recovery (Rosén and Lundborg 2000; Jerosch-Herold 2005). Dynamic detection thresholds can be assessed using tuning forks and vibrometers; however, these tests produce problems with standardization of testing technique (Bell-Krotoski et al. 1993; Rosén 1996).
2. **Discriminative tests:** Once a patient is able to discern touch spatial “discrimination” tests can be commenced. Of which there are a variety. Two-point discrimination (2PD) is the most commonly reported test within the literature and is incorporated into the Medical Research Council (MRC) score of sensory recovery (Mackinnon and Dellon 1988). However, the responsiveness of the 2PD test has been shown to be poor and its validity questioned (Jerosch-Herold et al. 2006). A second example of discrimination is Locognosia; the ability to localize touch. Locognosia requires the communication of the peripheral end-organ with the somatosensory cortex of the brain. An assessment of localization of touch within the hand has been developed and found to be a reliable and valid method for assessing spatial discrimination with median and/or ulnar injuries of the hand (Jerosch-Herold et al. 2006).
3. **Quantification tests:** Pertain to the ability to rank several objects and textures in degree of roughness, thickness, or weightiness. There is no formal validated testing technique. Clinically it is discerned through the use of functional activities.
4. **Identification tests:** In the final stage, sensation should be comprehensive enough to identify objects. Stereognosis relates to the ability of the hand to recognize objects in the absence of any visual clues. It is assessed using tactile gnosis tests which are arguably more clinically relevant than the simplistic assessments of touch described above; as they require both sensibility and motion. The Moberg pick-up test (see Sect. 5.2 below) is one example where everyday objects are identified with vision occluded (Moberg 1958). The Shape Texture Identification (STI) test is a valid and reliable instrument to when assessing recovery of the median and ulnar nerves (Rosén and Lundborg 1998; Jerosch-Herold et al. 2006). This assessment tool investigates the person’s ability to identify shapes and textures with the fingertips alone. The STI has had extensive research regarding

its validity, reliability and responsiveness with favorable results (Rosén et al. 2003; Jerosch-Herold et al. 2006).

The Rosen score, known as “The model instrument for documentation after nerve repair,” is a valid and reliable tool for monitoring change following median and ulnar nerve repair (Rosén and Lundborg 2000). It includes a multimodal assessment of sensation, motor function, cold intolerance and pain.

4.5 Sensory Re-education

Immediately after injury to the peripheral nerve the autonomous territory becomes devoid of sensation. Following this (usually within 24 h) the corresponding areas within the somatosensory cortex begin to shrink (Merzenich and Jenkins 1993). In addition, as a transected nerve regenerates, it is unlikely that all of the axons will regrow along their previous routes. If misdirection of growth occurs, it can lead to the target organs being reinnervated by different axons. Cortical functional reorganization is required in order for the brain to correctly interpret the information that it receives, allowing for a correct location of a stimulus.

Sensory re-education is a process whereby the brain is slowly reprogrammed in order to preserve and/or restore the sensory areas which have been affected by nerve injury (Jerosch-Herold 2011). Sensory retraining should commence immediately after peripheral nerve injury/repair (Lundborg et al. 2005). It can include techniques such as visualization, the use of alternate senses (such as vision or hearing), and the use of graded tactile stimuli (Jerosch-Herold 2011).

It is important that any program is tailored to the individual and considers their occupation and hobbies. Furthermore, the use of meaningful goal-directed activities within sensory programs promotes greater use of the hand and limb and increases the likelihood that the patient will engage with therapy (Byl et al. 2003; Callahan 2011).

4.5.1 Early Stages: Complete Sensory Loss

The patient should initially be advised to care for the denervated skin to avoid further injury such as pressure sores and burns.

When complete denervation has occurred, the aim within the early stages of recovery is to avoid, minimize, and modulate cortical reorganization (Rosén et al. 2003). It is well known that the brain has a multimodal capacity implying that specific neurones and associated cortical areas respond to stimuli provided by touch as well as vision and hearing (Bavelier and Neville 2002). Therefore, the use of other senses such as vision or sound can be used as a substitute for touch as a “sensory bypass strategy” (Jerosch-Herold 2011). Experimental studies have shown that viewing a body surface can directly enhance tactile perception and detection through activation of the somatosensory cortex (Avikainen et al. 2002; Grezes et al. 2003; Kuehn and Pleger 2018). This can occur even when the “touch” is not physical but a mirrored reflection (Kuehn and Pleger 2018; Moseley 2004). In addition, once the regeneration of sensory pathways to the skin has occurred, listening to the

friction sound of a hand being touched or touching various textures have also been shown to improve and expedite the patient's cognitive and adaptive response (Lundborg et al. 2005).

4.5.2 Later Stages: Intact Sensory Pathways

Once protective sensation is documented, classical sensory reeducation of tactile gnosis can commence (Bell-Krotoski et al. 1993). Treatment programs may begin with the gradual introduction of objects of different shapes and textures. They will progress onto training with regard to the localization of static and moving touch. If motor function allows, the use of different hand grips to manipulate, discriminate, and identify everyday objects can also be incorporated. Handedness is also an important consideration as it may influence functional recovery.

4.5.3 Desensitization

Hypersensitivity to touch can sometimes arise following nerve injury. Desensitization is the process of reducing hypersensitivity through the gradual relearning of "normal" response to sensory input (Imai et al. 1991). Desensitization begins by graded activity and exposure using a less irritating stimulus (e.g., the use of textures or immersion of the hand into differing particles such as sand or beads) progressing onto a more irritant stimulus (e.g., vibration) (Imai et al. 1991).

4.6 Pain

The International society of pain (IASP) define pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" (Merskey and Bogduk 1986). The experience of neuropathic pain is well documented following PNI (Birch 2011; Brown et al. 2018).

The complex nature of nerve injury and the effects that it has on the musculoskeletal system can often generate secondary soft tissue dysfunctions (e.g., edema, joint stiffness, and scar tissue). These give rise to pain which can be nociceptive and/or neuropathic in nature (Birch 2011). Neuropathic pain is known to affect outcomes and increase disability (Novak and von der Heyde 2013). Patient reported outcome measures (PROs) are well utilized to identify and monitor pain within a rehabilitation setting. The presence of neuropathic pain can be ascertained using the PainDetect tool (Freynhagen et al. 2006). Pain intensity is commonly assessed using Visual Rating Scale (VAS) and Numerical Rating Scale (NRS) (Delgado et al. 2018). However, the experience of pain goes well beyond this. Neuropathic pain is well known to manifest as sharp, sudden, shooting, electric, and burning in nature. These descriptors of pain can be assessed and recorded with tools such as the McGill Pain Questionnaire and the Brief Pain Inventory (Melzack 1975). Location and distribution of pain is also of importance and can be ascertained and monitored via the use of body charts.

The treatment of pain is challenging due to the psychological impact it can have on an individual. It is widely accepted that the input of talking therapies and in

particular psychology support can greatly influence pain perception and management (Wellington 2010; Janssen et al. 2019).

Medical management of pain is beyond the scope of this chapter. However, ensuring that patients are prescribed the correct level (and nature) of analgesia is of great importance thereby enabling physical therapy and rehabilitation to progress.

Several nonmedical pain management techniques exist. These include modalities such as sensory reeducation and Graded Motor Imagery (GMI). The use of “mirror visual feedback” is an element of GMI and has been reported to assist in alleviating pain symptoms experienced as a consequence of BPI and distal PNI (Giroux and Sirigu 2003; Mibu et al. 2016). The concept of this treatment approach was founded in phantom limb pain following amputation (Ramachandran and Altschuler 2009). The theory has been transferred and protocols for treatment developed in other neuropathic pathologies (Thurlow and Gray 2018).

Techniques such as mindfulness and relaxation have been widely publicized for their ability to reduce neuropathic pain (Bryce and Ragnarsson 2000; Fukushima et al. 2014). They are also widely employed within the management of PNI alongside task modification, activity pacing and the use of splints, supports, and orthoses (Janssen et al. 2019).

4.6.1 Transcutaneous Electrical Nerve Stimulation

Transcutaneous electrical nerve stimulation (TENS) is a common treatment for a range of pain conditions. A Cochrane review reported on the comparison between TENS and sham TENS in neuropathic pain states (Gibson et al. 2017). The quality of the published literature was found to be very low, rendering the authors unable to confidently state whether TENS is effective for pain control in people with neuropathic pain.

4.7 Cold Intolerance

Cold intolerance is a well-reported consequence of nerve injury. Cold Intolerance is present in approximately 80% of those who suffer an upper extremity PNI, with 20% developing severe or extreme symptoms (Irwin et al. 1997). Several studies have demonstrated the negative impact of cold intolerance on an individual's everyday life (Carlsson et al. 2010; Novak et al. 2012; Novak 2018). Symptoms include: pain and discomfort, altered sensibility, stiffness, and color change (Campbell and Kay 1998). There is a strong correlation between perceived problems from cold intolerance and a capacity to carry out daily activities (Rosén 1999).

Several assessment tools exist to screen for cold intolerance symptoms (Novak 2018). Patient reported questionnaires such as the Cold Sensitivity Scale and the Cold Intolerance Symptom Severity allow patients to rank their symptoms using a numeric scale (McCabe et al. 1991; Irwin et al. 1997). It is recognized that due to the complexity of cold intolerance these should be followed by further assessments relating cold sensitivity to everyday function and participation (Novak 2018).

4.8 Scar Tissue Formation

Scars are an inevitable consequence of surgery. The presence of scars can lead to psychological and physical impairments such as poor aesthetics and body image issues (Wellington 2010; Fearmonti et al. 2011). The main concern from a rehabilitation aspect is the possibility of the scar tissue forming adhesions which may restrict mobility. Furthermore, sensitivity within the scar can lead to pain, allodynia, and hypersensitivity.

Many scar assessment tools exist which are both patient and clinician reported. It has been suggested that scar features such as color, thickness, pliability, and surface area should all be considered. Examples of tools that consider these features are the Vancouver Scar Scale (Sullivan et al. 1990) and the Patient and Observer Scar Assessment Scale (Draaijers et al. 2004). The latter has been endorsed as it includes both clinician and patient reported measures (Verhaegen et al. 2011; Vercelli et al. 2009).

Scar management begins with patient education and scar massage. These can be commenced once the wound is healed and there are no signs of infection. Mobilization of the surrounding skin and joints is advocated to encourage the correct alignment of collagen fibers (Hardy and Woodall 1998; Fess and McCollum 1998). These interventions should be continued until the scar appears pale and inactive. If the scar is extensive, hypertrophied, keloid, or crosses a joint, further management techniques may need to be employed. These may include silicone gels, compression, ultrasound, and taping (Chang et al. 1995; Berchtold et al. 2016).

5 Activity and Participation

The Activity and Participation domain of the WHO-ICF covers a broad spectrum of experiences that one would consider to be everyday functional activities and life roles. These include activities of daily living such as personal care, meal preparation, feeding, socialization, and work. Therefore, the evaluation of function should include a meaningful measurement of the ability of a person to function or operate in their usual way. Patient and clinician reported outcome measures of function aim to gather information about how able an individual is to carry out everyday tasks. The information gained from these can help to monitor progress and prioritize specific treatment goals.

5.1 Patient Reported Outcomes

The Disabilities of the Arm Shoulder and Hand (DASH) is an upper limb functional assessment questionnaire which has been frequently used to evaluate outcome following PNI (Hudak et al. 1996; Novak et al. 2008, 2011; Dy et al. 2015). However, the questionnaire has come under criticism for not discriminating between hand dominance or injured versus non-injured limbs. Therefore, it is argued that

scores are not specific to a limb, rendering clinicians unable to determine whether the changes are related to nerve recovery or functional compensation (Hill et al. 2015).

Recently, a number of condition-specific PROs have been developed. The Impact of Hand Nerve Disorders (I-HAND) has been designed for use with individuals with PNI of the hand (Ashwood et al. 2018). The content of the I-HAND has been informed by exploratory interviews and includes 32 items covering impairment, pain, activity, and participation (Ashwood et al. 2018). Another notable condition-specific PROM is the BRachial Assessment Tool (BRAT), measuring activity in individuals with BPI (Hill et al. 2018a, b).

5.2 Clinician Reported Outcomes

The hand and specifically the fingers rely heavily on afferent feedback for sensory information to distinguish an object shape, mass, and the friction of the grasp that is necessary for fine motor control or dexterity. Therefore, any disruption of sensation to the fingers can compromise fine motor dexterity. Pure sensory tests, such as those for pressure threshold, pain, temperature, and vibration, do not accurately reflect hand function, which is also dependent on motor innervation and muscle function (Dellon and Kallman 1983). To fill this void a number of tests have been developed to quantify hand dexterity and grip function in activities of daily living.

The Moberg pick-up test (Moberg 1958) is a timed quick and inexpensive test which involves picking up, holding, manipulating, and identifying objects (coins, paperclips, small key, etc.) sighted and then blindfolded. The Sollerman Hand function test (Sollerman and Ejekskar 1995) is based on the concept that the prehensile movements of the human hand include variations of seven basic grips (pulp pinch, lateral pinch, tripod pinch, five finger pinch, diagonal, transvers, and spherical volar grip). This test gives a picture of both the ability and the quality of the hand grip.

Other commonly used functional tests include the Jebsen test, Nine-Hole Peg Test (NHPT), and the Functional Dexterity Test (FDT) (Jebsen et al. 1969; Kellor et al. 1971; Aaron and Jansen 2003). The Jebsen test uses standardized tasks to assess broad aspects of hand function commonly used in activities of daily living. The tasks are representative of various activities such as writing, turning pages, feeding, and picking up a range of objects. The test is commercially available, quick, and easy to administer. The NHPT and the FDT are pegboard tests which utilize accuracy and speed to measure dexterity (Aaron and Jansen 2003). The NHPT is the most widely used; however, the FDT has better psychometric properties when applied to an adult population (McPhee 1987; Schoneveld et al. 2009).

These functional tests have several limitations. Firstly, the quality of task execution is often not analyzed. Secondly, the tests are mostly unimanual and therefore may not represent normal function. Furthermore, there can be a learned effect with using the tests (Solari et al. 2005). Finally, it is worth noting that many of these tests were developed some decades ago and may not represent present-day function,

where society is becoming cashless, keyless, and the use of keyboards and touch screen devices are omnipresent.

The WHO-ICF encourages an assessment which relates to the individual's specific needs. Within BPI this may include commonly reported goals such as returning to work, driving, and sports and leisure activities (Brown et al. 2018). The diversity between individuals makes this aspect difficult to analyze using one single measure. Therefore tools that allow individual, progressive goal setting are well suited for this task. Two examples that are commonly used in a clinical setting are: the Canadian Occupational Performance Measure (Law et al. 1990) and the Patient Specific Functional Scale (Stratford et al. 1995). Both tools can be extremely effective when used to serially assess performance of everyday activities over a period of time, providing a meaningful, step-wise approach to an individual's return to societal roles and work.

5.3 Splinting/Supports/Orthoses

When splinting the hand following a nerve injury the motor and sensory function of the hand, purpose of the splint, and specific patient goals need to be considered. Different splints can be used depending on which nerve is affected.

Splints can assist in the maintenance of joint mobilization, tendon, and soft tissue length. Splinting may also address muscle imbalance within the hand by enabling the facilitation of the recovering weaker muscles (through the inhibition of stronger muscles) and/or substitute patterns of movement that are lost owing to denervated muscles. The main goal being to achieve a functional grasp which allows the individual to carry out activities of daily living, enabling the return to work and societal roles. Dynamic splints are recommended to maintain the normal pattern of movement while waiting for reinnervation (Colditz 2011).

5.3.1 Radial Nerve

The radial nerve innervates the extensors of the upper limb. Most radial nerve injuries will present following humeral or elbow fractures. Clinically, the patient presents with a wrist drop and an inability to extend the fingers and thumb.

Various splints can be provided ranging from a simple wrist splint (to maintain wrist extension leaving the fingers free to flex) to a complex dynamic splint with an outrigger component (to provide passive extension of the fingers/thumb while allowing active composite flexion; Fig. 3).

5.3.2 Median Nerve

The median nerve supplies the main forearm pronator, extrinsic long finger flexors, and thenar muscles. Injuries most commonly occur following trauma to the forearm. The patient will present with the inability to actively flex, abduct, or oppose the thumb and flex the index and middle fingers. There will also be a concurrent sensory deficit in the thumb, index, and middle fingers. The patient will find it difficult to use the hand for fine precision activities such as picking up coins, doing up buttons,

Fig. 3 Outrigger splint to facilitate the use of the hand and allow for objects to be gripped with the splint providing a passive release

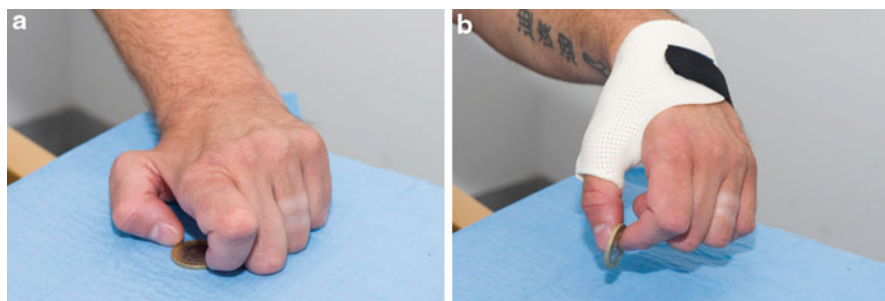
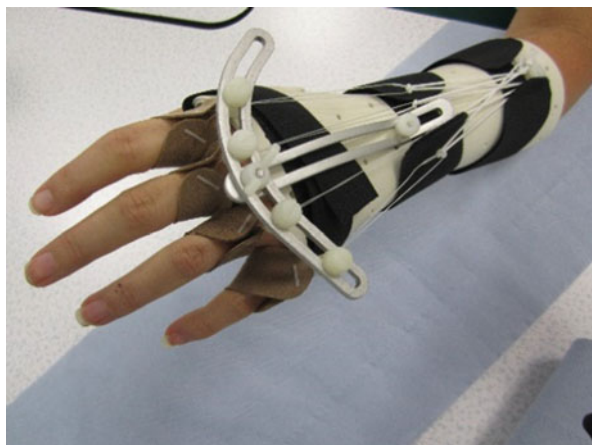


Fig. 4 A median nerve injury affecting the patient's ability to use their thumb to pinch and pick up a coin (a). Improved ability to perform this task is possible following provision of a static thumb opposition splint (b)

holding a pen, and gross functions like holding a cup/glass. Various splints can be provided to position the thumb in opposition and abduction in relation to the index and middle finger to improve function (Fig. 4a, b).

5.3.3 Ulnar Nerve

Injury to the ulnar nerve affects the small muscles of the hand. This causes an imbalance between the extrinsic long finger flexors and extensors and the intrinsic small muscles of the hand. There will also be a concurrent sensory deficit in the ring and little fingers. This leads to the appearance of “clawing” within the hand (Fig. 5a) and difficulty integrating the ring and little fingers into activities. The most prominent complaint is loss of dexterity and pinch/composite grip strength. Functional splints such as an anti-claw splint (Fig. 5b) can be provided to maintain balance and functional use of the hand while the nerve regenerates.

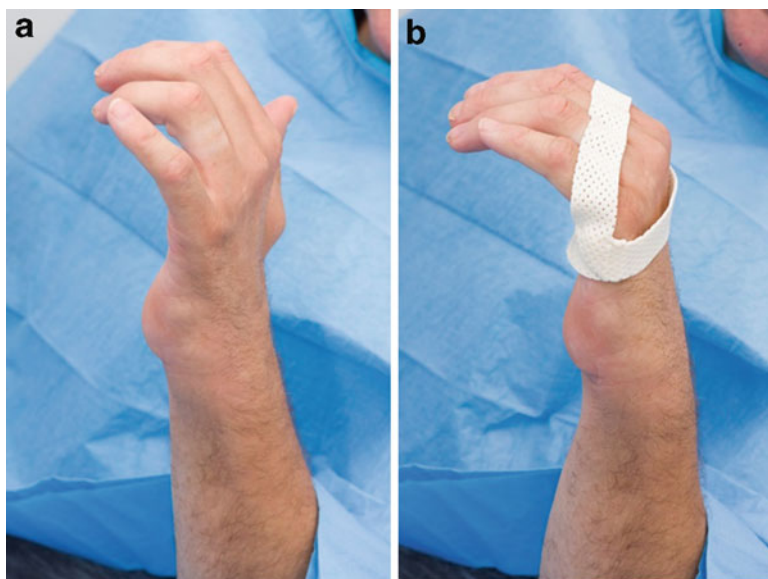


Fig. 5 “Clawing” of the hand caused by ulnar nerve injury (a). Anti-claw splint to give balance to the hand and enable function (b)



Fig. 6 Possible orthoses to assist with positioning of upper limb following BPI. (a) Elbow locking splint and (b) subluxation cuff

5.3.4 Brachial Plexus

Due to the length of time it may take for a nerve to regenerate proximally to distally BPI present with prolonged muscle imbalance and atrophy within the upper limb. Shoulder, elbow, and forearm supports and slings may be useful to assist with the functional positioning of the upper limb while awaiting recovery (Fig. 6).

6 Conclusions

A multidisciplinary approach is vital when assessing and treating patients in order to cater for their complex needs. The personal and societal impact of PNI are vast, with approximately 25% of patients with PNI (and up to 31–50% of patients with Brachial Plexus injuries) remaining out of employment at one and a half years post injury (Choi et al. 1997; Davis et al. 2011; Rasulić et al. 2017). Therefore, the role of the therapist is to enable any improvements in bodily functions to be translated into everyday living so that the individual's QoL can be fully optimized.

References

- Aaron DH, Jansen CWS (2003) Development of the functional dexterity test (FDT): construction, validity, reliability, and normative data. *J Hand Ther* 16(1):12–21
- al-Ghazal SK, Mc Kiernan M, Khan K, Mc Cann J (1994) Results of clinical assessment after primary digital nerve repair. *J Hand Surg Br* 19(2):255–257
- Al-Majed AA, Neumann CM, Brushart TM, Gordon T (2000) Brief electrical stimulation promotes the speed and accuracy of motor axonal regeneration. *J Neurosci* 20(7):2602–2608
- Anastakis DJ, Malesky MJ, Chen R, Davis KD, Mikulis D (2008) Cortical plasticity following nerve transfer in the upper extremity. *Hand Clin* 24(4):425–444
- Ashwood M, Jerosch-Herold C, Shepstone L (2017) Learning to live with a hand nerve disorder: a constructed grounded theory. *J Hand Ther* 43(8):864–874
- Ashwood M, Jerosch-Herold C, Shepstone L (2018) Development and validation of a new patient-reported outcome measure for peripheral nerve disorders of the hand, the I-HaND© Scale. *J Hand Surg Eur Vol* 43(8):864–874
- Avikainen S, Forss N, Hari R (2002) Modulated activation of the human S1 and S11 cortices during observation of hand actions. *NeuroImage* 15:640–646
- Bavelier D, Neville H (2002) Cross-modal plasticity. Where and how? *Nat Neurosci* 3:443–452
- Bell-Krotoski J, Weinstein S, Weinstein C (1993) Testing sensibility, including touch-pressure, two-point discrimination, point localization and vibration. *J Hand Ther* 6:114–123
- Berchtold M, Beckmann-Fries V, Meier Zürcher C (2016) Optimal scar management techniques for optimal hand function and aesthetics. *IFSSH Ezine* 6(1):19–23
- Bertelli JA, Ghizoni MF (2014) Nerve transfer from triceps medial head and anconeus to deltoid for axillary nerve palsy. *J Hand Surg Am* 39(5):940–947
- Birch R (2011) Pain. In: Birch R (ed) *Surgical disorders of the peripheral nerves*. Springer, London, pp 527–562
- Brawley LR, Culos-Reed SN (2000) Studying adherence to therapeutic regimens: overview, theories, recommendations. *Control Clin Trials* 21(5):S156–S163
- Brown H (2018) An international survey of the current use of electrical stimulation for adult traumatic brachial plexus injuries. Poster presentation, physiotherapy UK 2018, Birmingham. <https://innovations.csp.org.uk/sites/default/files/2019-01/FES%20Poster%20Physio%20UK%202018.pdf>
- Brown H, Johnson K, Gilbert A, Quick TJ (2018) The lived experience of motor recovery of elbow flexion following Oberlin nerve transfer: a qualitative analysis. *Hand Ther* 23(4):130–138
- Bryce TN, Ragnarsson KT (2000) Pain after spinal cord injury. *Phys Med Rehabil Clin* 11(1):157–168
- Byl NN, Nagajaran S, McKenzie AL (2003) Effect of sensory discrimination training on structure and function in patients with focal hand dystonia: a case series. *Arch Phys Med Rehabil* 84(10):1505–1514

- Callahan AD (2011) Chapter 14. Sensibility assessment for nerve lesions in continuity and nerve lacerations. In: Skirven TM, Lee Osterman A, Fedorczyk JM, Amadio PC (eds) *Rehabilitation of the hand and upper extremity*, 6th edn. Mosby, St. Louis
- Campbell DA, Kay SP (1998) What is cold intolerance? *J Hand Surg Br* 23(1):3–5
- Carlsson IK, Edberg AK, Wann-Hansson C (2010) Hand-injured patients' experiences of cold sensitivity and the consequences and adaptation for daily life: a qualitative study. *J Hand Ther* 23(1):53–62
- Carroll TJ, Herbert RD, Munn J, Lee M, Gandevia SC (2006) Contralateral effects of unilateral strength training: evidence and possible mechanisms. *J Appl Physiol* 101(5):1514–1522
- Chang C, Kuo Y, Chiu H, Lee J, Wong T, Jee S (1995) Hydration, not silicone, modulates the effects of keratinocytes on fibroblasts. *J Surg Res* 59(6):705–711
- Chemnitz A, Dahlin LB, Carlsson IK (2013) Consequences and adaptation in daily life – patients' experiences three decades after a nerve injury sustained in adolescence. *BMC Musculoskeletal Disord* 14:252
- Chen R, Asatakis DJ, Haywood CT, Mikulus DJ, Manktelow RT (2003) Plasticity of the human motor system following muscle reconstruction: a magnetic stimulation and functional magnetic resonance imaging study. *Clin Neurophysiol* 114(12):2434–2446
- Choi PD, Novak CB, Mackinnon SE, Kline DG (1997) Quality of life and functional outcome following brachial plexus injury. *J Hand Surg Am* 22(4):605–612
- Colditz JC (2011) Chapter 34. Splinting the hand with a peripheral nerve injury. In: Skirven TM, Osterman LH, Fedorczyk JM, Amadio PC (eds) *Rehabilitation of the hand and upper extremity*. Elsevier, Philadelphia
- Cram JR (2003) The history of surface electromyography. *Appl Psychophysiol Biofeedback* 28: 81–91. <https://doi.org/10.1023/A:1023802407132>
- Dahlin LB, Andersson G, Backman C, Svensson H, Björkman A (2017) Rehabilitation, using guided cerebral plasticity, of a brachial plexus injury treated with intercostal and phrenic nerve transfers. *Front Neurol* 8:72
- Davidson J (2004) A comparison of upper limb amputees and patients with upper limb injuries using the Disability of the Arm, Shoulder and Hand (DASH). *Disabil Rehabil* 26(14–15): 917–923
- Davis K, Taylor K, Anastakis K (2011) Nerve injury triggers changes in the brain. *Neuroscientist* 17 (4):407–422
- Delgado DA, Lambert BS, Boutris N, McCulloch PC, Robbins AB, Moreno MR, Harris JD (2018) Validation of digital visual analog scale pain scoring with a traditional paper-based visual analog scale in adults. *J Am Acad Orthop Surg Glob Res Rev* 2(3):e088
- Dellon AL (1991) Sensibility testing. In: *Operative nerve repair and reconstruction*. Lippincott, Philadelphia, pp 135–158
- Dellon AL, Kallman CH (1983) Evaluation of functional sensation in the hand. *J Hand Surg Am* 8:865–870
- Draaijers LJ, Tempelman FR, Botman YA, Tuinebreijer WE, Middelkoop E, Kreis RW, van Zuijlen PP (2004) The patient and observer scar assessment scale: a reliable and feasible tool for scar evaluation. *Plast Reconstr Surg* 113(7):1960–1965
- Dy CJ, Garg R, Lee SK, Tow P, Mancuso CA, Wolfe SW (2015) A systematic review of outcomes reporting for brachial plexus reconstruction. *J Hand Surg Am* 40(2):308–313
- Dyck PJ, Boes CJ, Mulder D, Milikan C, Windebank AJ, Dyck PJ, Espinosa R (2005) History of standard scoring, notation, and summation of neuromuscular signs. A current survey and recommendation. *J Peripher Nerv Syst* 10(2):158–173
- Fearmonti R, Bond J, Erdmann D, Levin LS, Pizzo SV, Levinson H (2011) The modified POSAS: a novel approach to defining pathologic and non-pathologic scarring. *Plast Reconstr Surg* 127 (1):242
- Fess EE (1995) Documentation: essential elements of an upper extremity assessment battery. In: Hunter JM, Mackin EJ, Callahan AD (eds) *Rehabilitation of the hand: surgery and therapy*, 4th edn. Mosby, St. Louis, pp 185–214

- Fess EE, McCollum M (1998) The influence of splinting on healing tissues. *J Hand Ther* 11(2): 157–161
- Fonseca MDCR, Elui VMC, Lalone E, da Silva NC, Barbosa RI, Marcolino AM, Ricci FPFM, MacDermid JC (2018) Functional, motor, and sensory assessment instruments upon nerve repair in adult hands: systematic review of psychometric properties. *Syst Rev* 7(1):175
- Freyenhagen R, Baron R, Gockel U, Tölle TR (2006) painDETECT: a new screening questionnaire to identify neuropathic components in patients with back pain. *Curr Med Res Opin* 22 (10):1911–1920
- Fu SY, Gordon T (1995) Contributing factors to poor functional recovery after delayed nerve repair: prolonged denervation. *J Neurosci* 5(5 Pt 2):3886–3895
- Fukushima FB, Bezerra DM, Boas PJ, Valle AP, Vidal EI (2014) Complex regional pain syndrome. *BMJ* 348:3683
- Gallese V, Fadiga L, Fogassi L, Rizzolatti G (1996) Action recognition in the premotor cortex. *Brain* 119(Pt 2):593–609
- Gibson W, Wand BM, O’Connell NE (2017) Transcutaneous electrical nerve stimulation (TENS) for neuropathic pain in adults. *Cochrane Database Syst Rev* 14(9):CD011976. <https://doi.org/10.1002/14651858>
- Giggins OM, Persson UM, Caulfield B (2013) Biofeedback in rehabilitation. *J Neuroeng Rehabil* 10:60. <https://doi.org/10.1186/1743-0003-10-60>
- Giraux P, Sirigu A (2003) Illusory movements of the paralyzed limb restore motor cortex activity. *NeuroImage* 20:S107–S111
- Gordan AM, Huxley AF, Julian FJ (1966) The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J Physiol* 184(1):170–192
- Gordon T, Stein RB, Martin T (1990) Physiological and histochemical changes in human muscle produced by electrical stimulation after spinal cord injury. *J Neurol Sci* 98(Suppl):141
- Goswami R, Anastakis D, Katz J, Davis K (2016) A longitudinal study of pain, personality, and brain plasticity following peripheral nerve injury. *Pain* 157(3):729–739
- Gray B (2016) Quality of life following traumatic brachial plexus injury: a questionnaire study. *Int J Orthop Trauma Nurs* 22:29–35
- Grezes J, Armony JL, Rowe J, Passingham RE (2003) Activations related to “mirror” and “canonical” neurones in the human brain: an fMRI study. *NeuroImage* 18:928–937
- Guttmann E, Guttmann L, Medawar PB, Young JZ (1942) The rate of regeneration of nerve. *J Exp Biol* 19:14–44
- Hardy M, Woodall W (1998) Therapeutic effects of heat, cold, and stretch on connective tissue. *J Hand Ther* 11(2):148–156
- Hill B, Williams G, Olver J, Bialocerkowski A (2015) Letter regarding outcome reporting for brachial plexus reconstruction. *J Hand Surg Am* 40(7):1504
- Hill B, Williams G, Olver J, Ferris S, Bialocerkowski A (2018a) Psychometric evaluation of the brachial assessment tool. Part 1: reproducibility. *Arch Phys Med Rehabil* 99(4):629–634
- Hill B, Williams G, Olver J, Ferris S, Bialocerkowski A (2018b) Preliminary psychometric evaluation of the brachial assessment tool. Part 2: construct validity and responsiveness. *Arch Phys Med Rehabil* 99(4):736–742
- Hill J, Turner LC, Jones RD, Jimulia T, Miller C, Power D (2019) The stages of rehabilitation following motor nerve surgery. *J Musculoskelet Surg Res* 3(1):60–67
- Hudak PL, Amadio PC, Bombardier C (1996) Development of an upper extremity outcome measure: the DASH (disabilities of the arm, shoulder, and head). *Am J Ind Med* 29(6):602–608
- Hundepool CA, Ultee J, Nijhuis TH, Hout P, Jaquet JB, Spauwen PHM, Hovius SE (2015) Prognostic factors for outcome after median, ulnar, and combined median–ulnar nerve injuries: a prospective study. *J Plast Reconstr Aesthet Surg* 68(1):1–8
- Imai H, Tajima T, Natsumi Y (1991) Successful re-education of functional sensibility after median nerve repair at the wrist. *J Hand Surg Am* 16(1):60–65
- Immordino-Yang MH (2008) The smoke around mirror neurons: goals as sociocultural and emotional organizers of perception and action in learning. *Mind Brain Educ* 2(2):67–73

- Irwin MS, Gilbert SEA, Terenghi G, Smith RW, Green CJ (1997) Cold intolerance following peripheral nerve injury: natural history and factors predicting severity of symptoms. *J Hand Surg Br* 22(3):308–316
- Janssen RM, Satink T, Ijspeert J, van Alfen N, Groothuis JT, Packer TL, Cup EH (2019) Reflections of patients and therapists on a multidisciplinary rehabilitation programme for persons with brachial plexus injuries. *Disabil Rehabil* 41(12):1427–1434
- Jebsen RH, Taylor NEAL, Trieschmann RB, Trotter MJ, Howard LA (1969) An objective and standardized test of hand function. *Arch Phys Med Rehabil* 50(6):311–319
- Jerosch-Herold C (2005) Assessment of sensibility after nerve injury and repair: a systematic review of evidence for validity, reliability and responsiveness of tests. *J Hand Surg Br* 30(3):252–264
- Jerosch-Herold C (2011) Sensory relearning in peripheral nerve disorders of the hand: a web-based survey and Delphi consensus method. *J Hand Ther* 24(4):292–299
- Jerosch-Herold C, Rosén B, Shepstone L (2006) The reliability and validity of the locognosia test after injuries to peripheral nerves in the hand. *J Bone Joint Surg Br* 88(8):1048–1052
- Kahn LC, Moore A (2016) Donor activation focused rehabilitation approach. Maximising outcome after nerve transfers. *Hand Clin* 32:263–277
- Kellor M, Frost J, Silberberg N (1971) Hand strength and dexterity. *Am J Occup Ther* 25:77–83
- Kendall F, McCreary E (1983) *Muscles testing and function*, 3rd edn. Williams and Wilkins, Philadelphia, pp 10–11
- Kern H, Hofer C, Mödlin M, Forstner C, Raschka-Högler D, Mayr W, Stöhr H (2002) Denervated muscles in humans: limitations and problems of currently used functional electrical stimulation training protocols. *Artif Organs* 26(3):216–218
- Khan R, Birch R (2001) Iatrogenic injuries of peripheral nerves. *J Bone Joint Surg Br* 83(8):1145–1148
- Kim JH (2017) The effects of training using EMG biofeedback on stroke patients upper extremity functions. *J Phys Ther Sci* 29:1085–1088. <https://doi.org/10.1589/jpts.29.1085>
- Kuehn E, Pleger B (2018) How visual body perception influences somatosensory plasticity. *Neural Plast* 2018(4):1–12
- Law M, Baptiste S, McColl M, Opzoomer A, Polatajko H, Pollock N (1990) The Canadian occupational performance measure: an outcome measure for occupational therapy. *Can J Occup Ther* 57(2):82–87
- Lee JY, Kircher MF, Spinner RJ, Bishop AT, Shin AY (2012) Factors affecting outcome of triceps motor branch transfer for isolated axillary nerve injury. *J Hand Surg Am* 37(11):2350–2356
- Lundborg G, Björkman A, Hansson T, Nylander L, Nyman T, Rosén B (2005) Artificial sensibility of the hand based on cortical audiotactile interaction: a study using functional magnetic resonance imaging. *Scand J Plast Reconstr Surg Hand Surg* 39(6):370–372
- MacAvoy MC, Green DP (2007) Critical reappraisal of Medical Research Council muscle testing for elbow flexion. *J Hand Surg Am* 32(2):149–153
- Mackinnon S, Dellon A (1988) *Surgery of the peripheral nerve*. Thieme Publishing Group, New York
- Masterson J (2015) The role of emotion, vision and touch in movement learning neuroplasticity and the mirror neuron system. *J Psychol Clin Psychiatry* 3(5):00149
- McCabe SJ, Mizgala C, Glickman L (1991) The measurement of cold sensitivity of the hand. *J Hand Surg Am* 16A:1037–1040
- McDermid J, Solomon G, Valdes K, American Society of Hand Therapists (2015) *Clinical assessment recommendations*, 3rd edn. American Society of Hand Therapists, New York/Mont Laurel
- McDonald J, Pettigrew J (2014) Traumatic brachial plexus injury: the lived experience. *Br J Occup Ther* 77:147–154
- McGlone F, Reilly D (2010) The cutaneous sensory system. *Neurosci Biobehav Rev* 34(2):148–159
- McPhee SD (1987) Functional hand evaluations: a review. *Am J Occup Ther* 41(3):158–163

- Medical Research Council (1943) Aids to the investigation of the peripheral nervous system. Her Majesty's Stationary Office, London
- Melzack R (1975) The McGill Pain Questionnaire: major properties and scoring methods. *Pain* 1(3):277–299
- Merskey H, Bogduk N (1986) Classification of chronic pain. Descriptions of chronic pain syndromes and definitions of pain terms. Prepared by the International Association for the Study of Pain, Subcommittee on Taxonomy. *Pain Suppl* 3:S1–S226
- Merzenich MM, Jenkins WM (1993) Reorganization of cortical representations of the hand following alterations of skin inputs induced by nerve injury, skin island transfers, and experience. *J Hand Ther* 6(2):89–104
- Mibu A, Nishigami T, Tanaka K, Osumi M, Tanabe A (2016) Successful graded mirror therapy in a patient with chronic deafferentation pain in whom traditional mirror therapy was ineffective: a case report. *Pain Pract* 16(4):E62–E69
- Miller C, Peek AL, Power D, Heneghan NR (2017) Psychological consequences of traumatic upper limb peripheral nerve injury: a systematic review. *Hand Therapy* 22(1):35–45
- Moberg E (1958) Objective methods for determining the functional value for sensibility of the hand. *J Bone Joint Surg Br* 40:454–476
- Moorhead J, Cooper C, Moorhead P (2011) Personality type and patient education in hand therapy. *J Hand Ther* 24(2):147–154
- Mooventhan A, Nivethitha L (2014) Scientific evidence-based effects of hydrotherapy on various systems of the body. *N Am J Med Sci* 6(5):199–209. <https://doi.org/10.4103/1947-2714.132935>
- Moseley L (2004) Graded motor imagery is effective for long standing complex regional pain syndrome: a randomised controlled trial. *Pain* 108:192–198
- Moseley L (2012) The graded motor imagery handbook. Noigroup Publications, Adelaide
- Neblett R (2016) Surface electromyographic (SEMG) biofeedback for chronic low back pain. *Healthcare* 4(2):27
- Neumayer C, Happak W, Kern H, Gruber H (1997) Hypertrophy and transformation of muscle fibers in paraplegic patients. *Artif Organs* 21(3):188–190
- Novak CB (2008) Rehabilitation following motor nerve transfers. *Hand Clin* 24(4):417–423
- Novak CB (2018) Cold intolerance after nerve injury. *J Hand Ther* 31(2):195–200
- Novak CB, von der Heyde RL (2013) Evidence and techniques in rehabilitation following nerve injuries. *Hand Clin* 29:383–392
- Novak CB, von der Heyde RL (2015) Rehabilitation of the upper extremity following nerve and tendon reconstruction: when and how. *Semin Plast Surg* 29:73–80
- Novak CB, Anastakis DJ, Beaton DE, Katz J (2008) Patient-reported outcome after peripheral nerve injury. *J Hand Surg Am* 34(2):281–287
- Novak CB, Anastakis DJ, Beaton DE, Mackinnon SE, Katz J (2011) Biomedical and psychosocial factors associated with disability after peripheral nerve injury. *J Bone Joint Surg Am* 93(10):929–936
- Novak CB, Anastakis DJ, Beaton DE, Mackinnon SE, Katz J (2012) Cold intolerance after brachial plexus nerve injury. *Hand* 7(1):66–71
- Pankow D, Glatzel W, Grimm I, Ponsold W, Tietze K (1974) Effect of carboxy- or methemoglobinemia on motor conduction velocity. *Experientia* 30(9):1057–1058
- Quick TJ, Singh AK, Fox M, Sinisi M, MacQuillan A (2016) A quantitative assessment of the functional recovery of flexion of the elbow after nerve transfer in patients with a brachial plexus injury. *Bone Joint J* 98B(11):1517–1520
- Ramachandran VS, Altschuler EL (2009) The use of visual feedback, in particular mirror visual feedback, in restoring brain function. *Brain* 132(7):1693–1710
- Ramachandran VS, Rogers-Ramachandran D (1996) Synaesthesia in phantom limbs induced with mirrors. *Proc Biol Sci* 263(1369):377–386
- Rasulić L, Savić A, Živković B, Vitošević F, Mićović M, Baščarević V, Mandić-Rajčević S (2017) Outcome after brachial plexus injury surgery and impact on quality of life. *Acta Neurochir* 159(7):1257–1264

- Rinker B, Fink BF, Barry NG, Fife JA, Milan ME, Stoker AR, Nelson PT (2011) The effect of cigarette smoking on functional recovery following peripheral nerve ischemia/reperfusion injury. *Microsurgery* 31(1):59–65
- Rosén B (1996) Recovery of sensory and motor function after nerve repair: a rationale for evaluation. *J Hand Ther* 9(4):315–327
- Rosén B (1999) Cold intolerance in the hand – an unsolved problem. *Br J Hand Ther* 4(1):23–25
- Rosén B, Lundborg G (1998) A new tactile gnosis instrument in sensibility testing. *J Hand Ther* 11(4):251–257
- Rosén B, Lundborg G (2000) A model instrument for the documentation of outcome after nerve repair. *J Hand Surg Am* 25(3):535–543
- Rosén B, Balkeniu C, Lundborg G (2003) Sensory re-education today and tomorrow: a review of evolving concepts. *Br J Hand Ther* 8(2):48–56
- Rothgangel A, Braun S, Beurskens A, Seitz R, Wade D (2011) The clinical aspects of mirror therapy in rehabilitation. *Int J Rehabil Res* 34(1):1–13
- Schoneveld K, Wittink H, Takken T (2009) Clinimetric evaluation of measurement tools used in hand therapy to assess activity and participation. *J Hand Ther* 22(3):221–236
- Seddon HJ, Medawar PB, Smith H (1943) Rate of regeneration of peripheral nerves in man. *J Physiol* 102(2):191–215
- Solari A, Radice D, Manneschi L (2005) The multiple sclerosis functional composite: different practice effects in the three test components. *J Neurol Sci* 228:71–74
- Sollerman C, Ejekkar A (1995) Sollerman hand function test: a standardised method and its use in tetraplegic patients. *Scand J Plast Reconstr Surg Hand Surg* 29:167–176
- Stratford P, Gill C, Westaway M, Binkley J (1995) Assessing disability and change on individual patients: a report of a patient specific measure. *Physiother Can* 47(4):258–263
- Sturma A, Hruba LA, Prahm C, Mayer JA, Aszmann OC (2018) Rehabilitation of upper extremity nerve injuries using surface EMG biofeedback: protocols for clinical application. *Front Neurosci* 12:906
- Sullivan TA, Smith J, Kermode J, McIver E, Courtemanche DJ (1990) Rating the burn scar. *J Burn Care Rehabil* 11(3):256–260
- Thurlow G, Gray B (2018) Complex regional pain syndrome. *Int J Orthop Trauma Nurs* 30:44–47
- Trojaborg W (1970) Rate of recovery in motor and sensory fibres of the radial nerve: clinical and electrophysiological aspects. *J Neurol Neurosurg Psychiatry* 33(5):625–638
- Vercelli S, Ferriero G, Sartorio F, Stissi V, Franchignoni F (2009) How to assess postsurgical scars: a review of outcome measures. *Disabil Rehabil* 25(31):2055–2063
- Verdú E, Ceballos D, Vilches JJ, Navarro X (2000) Influence of aging on peripheral nerve function and regeneration. *J Peripher Nerv Syst* 5(4):191–208
- Verhaegen PD, van der Wal MB, Middelkoop E, van Zuijlen PP (2011) Objective scar assessment tools: a clinimetric appraisal. *Plast Reconstr Surg* 127(4):1561–1570
- Verma CV, Vora T, Thatte M, Yardi S (2019) Patient perception after traumatic brachial plexus injury: a qualitative case study. *J Hand Ther* 24(2):55–61
- Vikström P, Rosén B, Carlsson IK, Björkman A (2018) The effect of early relearning on sensory recovery 4 to 9 years after nerve repair: a report of a randomized controlled study. *J Hand Surg Eur Vol* 43(6):626–630
- Wellington B (2010) Quality of life issues for patients following traumatic brachial plexus injury – part 2 research project. *Int J Orthop Trauma Nurs* 14:5–11
- Wilcox M, Brown H, Johnson K, Sinisi M, Quick TJ (2019) An assessment of fatigability following nerve transfer to reinnervate elbow flexor muscles. *Bone Joint J* 101B(7):867–871
- World Health Organisation (2001) International Classification of Functioning, Disability, and Health (ICF). World Health Organisation, Geneva
- Wu P, Chawla A, Spinner RJ, Yu C, Yaszemski MJ, Windebank AJ, Wang H (2014) Key changes in denervated muscles and their impact on regeneration and reinnervation. *Neural Regen Res* 9(20):1796

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